Population Genetics of Bush-encroaching *Acacia mellifera* at Pniel, Northern Cape Province, South Africa

by

Beka Jeremia Nxele

Thesis presented in partial fulfillment of the requirements for the degree Master of Science (Conservation Ecology) at the University of Stellenbosch

Supervisor: Dr. Shayne M. Jacobs

Faculty of AgriSciences

Department of Conservation Ecology

December 2010

Declaration

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2010

ABSTRACT

Two populations of *Acacia mellifera* were noted in Pniel, which is a semi-arid savanna, near Kimberley in the Northern Cape province of South Africa. One population appeared on a rocky, andesitic laval ridges (soil pH_{KCL} 6.5-7.0) along the Vaal river. The other appeared in a sandveld area (soil pH_{KCL} 4). Bush encroachment by *A. mellifera* was found to be more extensive on the rocky areas than in the sandveld and the two habitats differed extensively on soil pH, clay and silt contents and also on water holding capacities. The rocky habitat was thus deduced to have a higher CEC. Seeds were sampled on a logarithmic scale for allozyme analysis and also randomly in each of the two habitats for local adaptation tests, in which case lime ($CaCO_3$) and organic matter (cow-dung) were used in a completely-crossed design. Detected interaction effects (between population source and pH; population source and organic matter and between pH and organic matter) and significant differences could not separate the two populations as the differences occurred across populations.

Random genetic differences leading to phenotypic plasticity in the two observed populations, might be responsible for the observed phenotypic differences. Allozymic data showed no significant differences between the two populations and the genetic distance between and within the populations also confirmed that the two populations had not genetically differentiated. The Mantel Test on the two populations, showed nonsignificant results. Nei's UPGMA dendrogram revealed that the game farm subpopulations were more primitive and genetically related to each other. Despite differences in allozyme frequencies, between the sampled sites, genetic differentiation was found to be low (F_{ST} = 0.337). Nei's (1972) original measures of genetic distance ranged between 0.871 and 1.000 with a mean of 0.949 ± 0.053. The study concluded that the two observed populations had not genetically differentiated and no local adaptation could be established rather phenotypic plasticity was

evident and resulted in the observed divergent growth forms. Nonetheless, the overall direction of spread of encroachment appeared to be the eastward.

Key words: *Acacia mellifera*, bush encroachment, population differentiation, allozymes, local adaptation, phenotypic plasticity.

OPSOMMING

Twee bevolkings van *Acacia mellifera* is gevind in Pniel, wat ‘n semi-ariede savanna is naby Kimberley in die Noord-Kaap provinsie van Suid-Afrika. Een bevolking het voorgekom op klipperige andesitiese lava riwwe (grond pH_{KCL} 6.5-7.0) al langs die Vaalrivier. Die ander het voorgekom in ‘n sandveld area (soil pH_{KCL} 4). Bos-oorskryding deur *A. mellifera* was meer uitgebreid op die klipperige areas as in die sandveld en die twee habitats het noemenswaardig verskil ten opsigte van grond pH, klei en silt inhoud asook waterhoukapasiteit. Dit kan was dus afgelei word dat die klipperige habitat ‘n hoër CEC het. Die sade was versamel op ‘n logaritmiese skaal vir allosiem-analise en ook ewekansig in die twee habitats vir lokale aanpassings toetse. In dié gevalle was kalk ($CaCO_3$) en organiese material (koeimis) gebruik in ‘n totaal-gekruisde ontwerp. Bespeurde interaksie effekte (tussen bevolkings bron en pH; bevolkings bron en organiese material en tussen pH en organiese material) en noemenswaardige verskille kon nie die twee bevolkings skei nie, aangesien die verskille voorgekom het regdeur die twee bevolkings.

Ewekansige genetiese verskille wat lei tot fenotipiese plastisiteit tussen die twee waargeneemde bevolkings mag dalk verantwoordelik wees vir die waargeneemde fenotipiese verskille. Allosiem-data het geen beduidende verskille gelewer tussen die twee bevolkings nie en genetiese afstand binne en tussen die bevolkings het ook bevestig dat die twee bevolkings nie geneties gedifferensiëer is nie. Die Mantel toets op die twee bevolkings het geen beduidende resultate gelewer nie. Nei se UPGMA dendogram get gewys dat die wildsplaas bevolkings was meer primitief en geneties verwant aan mekaar. Ten spyte van die allosiem frekwensies tussen die gemonsterde gebiede, was die genetiese differensiasie laag (F_{ST} = 0.337). Nei (1972) se oorspronlike meeting van genetiese afstand het tussen 0.871 en 1.000 beloop met ‘n gemiddeld van 0.949 ± 0.053. Die studie het bepaal dat die twee

waargeneemde bevolkings nie geneties gedifferensiëer het nie en dat geen lokale aanpassing teenwoordig was nie. Fenotipiese plastisiteit was duidelik waarneembaar en het gelei tot die divergerende groeivorme. Nieteenstaande, was die algehele rigting van oorskryding ooswaarts.

Sleutel woorde: Acacia mellifera, bos-oorskryding, bevolkings-differensiasie, allosieme, lokale aanpassing, fenotipiese plastisiteit.

ACKNOWLEDGEMENTS

I would like to pass my sincere gratitude to all those who contributed, in one way or another, towards the accomplishment of this project:

To my supervisor Dr. Shayne Jacobs for his leadership, commitment, guidance and willingness to adopt me as one his students after my original supervisor had to change academic institutions. Also for his reassurance, solace and encouragement. My ex-supervisor Professor David Ward for his dedication, commitment, and passion for the subject which he developed in me and for his assistance with my work throughout the years of my study. Vernon for sponsoring with Malmesbery sand for my Greenhouse experiment. My wife Thembeka Nxele for the love and support that she gave me through my road accident in Stellenbosch and for giving me hope that "*the light will shine*". I shall ever remain grateful to you, LuZuko and Yamkela. Finally, the National Research Foundation (NRF), Cape Tercentenary, Erickssen & Ethel Trust Foundation and the University of Stellenbosch for financial aid.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

Chapter 1

GENERAL INTRODUCTION

1.1 Introduction

Savannas occupy the majority of the surface area of Southern Africa, yet there exist a general lack of information on the genetic diversity of savanna tree species, especially those that encroach savannas such as *Acacia mellifera*. Thus controlling bush-encroachment remains a challenge, and this is exacerbated by a lack of information on the factors that cause bush-encroachment. Encroachment by *Acacia mellifera* is widespread in many parts of Southern Africa (Moleele *et al.* 2002; Smit, 2004). The species can reproduce both vegetatively and sexually, and is therefore difficult to control once it encroaches (Adams, 1967). Knowing under what conditions the plant will switch from one mode of reproduction to the other or by which mode of reproduction an encroaching population is propagating might be very important in structuring control measures. For instance, if the population is engaging in vegetative reproduction, application of biological- and chemical controls might be a waste of both money and time whereas it might prove more effective with sexual reproduction.

Bush-encroachment has become a major management issue for conservation agencies, public and private landowners alike; some introduce bio-control and chemical measures to halt and reverse the spread of, especially, leguminous savanna trees and shrubs (Radford *et al.* 2002). The application of bio-controls might be effective to a certain extent with some *Acacia* species because acacias are also known to be both phenotypically and genotypically variable (Shrestha *et al.* 2002). With that being the case, it can be expected that different genotypes will respond differently to a particular biological control treatment. Thus, with the application of bio-control treatments, it becomes of paramount importance to treat each population as a separate entity and to obtain the basic information on genetic variability for a successful control.

1.2 Savannas

At a continental scale, savannas are regarded as the most dominant biome and also provide a livelihood to a major part of the human population of Africa (Scholes & Walker, 1993). According to Scholes (1997), savannas amount to 54% of southern Africa, some 1 435 713 km^2 is occupied by open or closed canopy savannas. Savannas may be conceptualized as biomes largely dominated by woody vegetation and grasses. Commonly, they are at least two-layered above the ground structure: viz, a discontinuous crown cover of the tree layer (2-10 m) and a grassy layer (0.5 – 2 m) (Scholes, 1997). Savannas generally consist of tropical vegetation in which C_4 grasses often dominate the herbaceous stratum, and a woody stratum which are usually fire-dominant and which ranges from low aerial cover to a closed woodland (Baruch & Bilbao, 1999; Magnusson *et al.* 1999). The former constitutes open savannas and the latter, closed savannas. Leguminous trees and shrubs, some of which has been shown to be nitrogen fixers, dominate the tree layer in many parts of Southern Africa (Van Auken, 2009).

A savanna environment could be described as hot, seasonally dry grassland with scattered trees and is mostly found to be intermediate between a grassland and a forest. Southern African savannas range from tall, moist woodlands receiving up to 1800 mm rainfall per year in northern Angola, to sparse grasslands with scattered thorn bushes on the margins of the Namib Desert where rainfall might be as low as 50mm during drought years (Scholes, 1997). Rainfall usually occurs in the warmer, summer months with a dry period of between two to eight month's duration during which fire is a typical phenomenon at intervals varying from one to fifty years (Huntley, 1982). Included within this concept are the miombo and mopane grasslands, the tall grass "derived savannas" bordering the Guineo-Congolian rainforests, the shrublands of the Kalahari and the Khomas Hochland, the grassy dambos and chanas of central Africa and the succulent thickets of the valley bushveld of the eastern Cape

(Huntley, 1982; Scholes, 1997). Such a diversity of physiognomy, flora and environmental conditions has tended to mask otherwise clear relationships between constituent ecosystems - relationships that indicate the existence of distinctive "arid" and "moist" savanna biomes in southern Africa (Scholes, 1997).

Arid and moist savannas differ significantly in terms of their floras and faunas, climatic and soil conditions, physiognomy and dynamics. The differences are easily recognized in parts of central Africa but which merge increasingly towards the south and south-east, ultimately forming a small-scale vegetation mosaic separated by subtle soil and climatic changes (Huntley, 1982; Scholes, 1997). According to Scholes *et al.* (2002), on the western front, where savannas gradually merge into deserts, it appears there is a clear gradient in woody biomass which might correlate with south to north gradient in rainfall (i.e. from 200 to 1000mm mean annual precipitation). Above the minimum level of 200mm, the woody basal area increases at a rate of about 2.5m/ha/100mm, whilst the mean maximum height also increases reaching 20m at about 800mm mean annual precipitation (Scholes *et al.* 2002). Furthermore, the number of tree species contributing to more than 95% of the woody basal area, increase from one at 200mm to 16 at 1000mm and it is members of the Mimosaceae (mainly Acacia) that dominate the tree layer up to 400mm (Scholes *et al.* 2002). The structural variation noted within savannas and the differences observed between savannas illustrate the role and importance of these biomes in the ecosystem in that they provide diverse environments for diverse species, (Teague & Smit, 1992; van der Vijver *et al.* 1999). Savannas provide a livelihood to many, primarily through supplying grazing areas, fuel wood, timber and other resource contributions to the informal and subsistence economies (Scholes, 1997). They are the main locations and supporters of livestock and ecotourism industries; they have a global contribution through their emissions of trace gases from soils, fires, vegetation and animals (Otter *et al.* 2002); they sequestrate carbon in their soils and

biomass (Hernández-Hernández & López-Hernández, 2002), and host a reasonable degree of biodiversity.

Southern African savannas are regarded as part of the Sudano-Zambezian phytochorion as they display many common genera and species with the savannas of Central and East Africa (Scholes, 1997). With comparison to the savannas of West Africa, they share many families but very few species (Scholes, 1997). Despite this continental variation, some common features do exist between southern African savannas and those of the Indian Peninsula, though fewer with those of America, Australia or South-East Asia (Johnson & Tothill, 1985). However, this floristic differentiation does not take away the common essence of a savanna. Global savannas still have some commonality, they are presumed to share similar structural dynamics and function (Otter *et al.* 2002).

1.3 Where are Savannas Situated?

The sensitivity of savannas to mismanagement, their global distribution and the amount of biodiversity they nurture, as well as the number of human populations they support, is good enough motivation to think of savannas as worth protecting. Land-use change is, without doubt, one of the most important factors affecting ecological systems and also interacts with other components in causing global change (Vitousek, 1992; Ringrose *et al.* 1998; Manlay *et al.* 2002). As such, savanna systems that are subjected to some form of anthropogenic activity are prone to disruption.

Savannas are found in Africa, Madagascar (an island off the East Coast of Africa), Australia, South America, India, and the Myanmar-Thailand region of Southeast Asia (Johnson & Tothill, 1985). Although they seem to be evenly distributed within the globe, their distribution is notably bound within the tropical belt (Hutley & Setterfield, 2008). They extend across the dry tropics through to the subtropics, and most often where they are found,

it is most common that they are bordering a rainforest (Johnson & Tothill, 1985). In most cases they have an extended dry season and a rainy season.

Due to this pronounced seasonal variation, the animals that are found in savannas can be seen to have adapted to a great deal of variability in the food supply throughout the year. This adaptation could be because there are times of plenty (during and after the wet season) and times of scarcity (during the dry season). In order to cope with life in savannas, some animals have opted for migration during the dry seasons. Prominent animal taxa found in savanna range from invertebrates (like grasshoppers, termites, and beetles) to mega-herbivores and subsequently, predators and from this, it can be noted that savannas of the world, as different as they are, also support a significant amount of faunal diversity.

In Africa, savannas largely cover most parts of the continent and have considerable amount of structural variation in terms of tree-grass densities and also a significant amount of biodiversity. This variation may be affected by factors such as rainfall frequency and disturbance for example, grazing, fires, habitat destruction (in the case of elephants) (van de Vijver *et al.* 1999; Sankaran *et al.* 2005). Grasses, which have shallow root systems and thus can utilize topsoil nutrients and water (Walter, 1939; Huston, 1994), usually outcompete the woody plants (which have deep penetrating roots and are slow growing). Such massive germination of woody plants transforms open, diversity-rich savannas into closed, unusable savannas, which have low biodiversity. The potential of the land to sustain both humans and their livestock is thus reduced and biodiversity is negatively affected (Asner *et al*. 2004).

1.4 Local Adaptation

In order for species to survive, it becomes imperative for them to adapt to their environment. This creates habitats for other species, which might be associated with the species undergoing local adaptation. Therefore one species' response to an environmental condition might result

in a creation of a habitat, which might later result in inter- and intra-competition between and within species because of the resources that might be available in that particular habitat (Magnusson *et al.* 1999). Extensive empirical work has demonstrated that local adaptation exists across different taxa, both in the Animal and Plant Kingdoms (Raven & Johnson, 1992). A sound understanding of local adaptation might be a tool for understanding why and how speciation occurs.

Local adaptation may be viewed as a gene by environment interaction between a species and its environment which enables the species to respond to an environmental stimulus, appropriately, for a specific environmental condition (Schlichting, 1986; de Jong, 1990) rather than an epiphenomenon which is due to resource availability. Although local adaptation has frequently been documented, there is controversy over the spatial scale of adaptive evolution (Fenster *et al.* 1997). In a study by Fenster *et al.* (1997) on evaluating adaptive differentiation between populations of the annual legume *Chamaecrista fasciculata*, using a replicated common-garden design (complete-cross design); it was found that metapopulation processes and spatial environmental variation (e.g. changes in soil quality) act together to increase local adaptation, except over great distances.

Therefore, local adaptation may be visualized as a product of the strength of the two factors (metapopulation processes & spatial environmental variation) combined. Where their combined effect is non-significant, local adaptation would range from non-existent to poor whereas where the combined effects are significant, local adaptation will be strongest. With the effect of distance, Fenster *et al.* (1997) suggest that even though there is little understanding on the frequency with which epistasis (gene interaction) contributes to the evolution of natural populations, both selection and drift contribute to population differentiation that is based on epistatic genetic divergence.

Finally, the mismanagement of savannas does not only affect the vegetation, which directly affects biodiversity but also threatens their very identity. Therefore, biological diversity requires protection from all levels, i.e. from the genome to the ecosystem (De Groot *et al.* 2003). Should local adaptation be prevalent (in the study area), that is if a source population is only dominant in its original site (e.g. rocky or sandveld), this would imply that the two populations are different. Thus, a biological treatment for one population might not necessarily be as effective as on the other. Adopting fire as a treatment might be beneficial for it is non-selective. However, fire will uniformly burn either population including everything else that is combustible and that includes the grasses. Bearing in mind grasses could have a role in encroachment in that their absence in the system might be advantageous for encroachment. Therefore, aspects such as the relative role of fire intensity, timing and frequency (Radford *et al.* 2001; Danthu *et al.* 2003) need to be borne in mind should fire be used as a control treatment.

1.5 Contributions of Molecular Genetics to Conservation

Molecular genetics in ecology is a recent advance and it allows scientists to study and explore populations, at an in-depth detail and at a larger scale, without the study being hazardous to the concerned population. In other words, it is regarded as a non-invasive approach through which there is less interference with wild populations for instance, as there is less need of catching individuals especially animals (Taberlet & Luikart, 1999). This advantage further accommodates research to be undertaken even in small populations which might be at the verge of extinction (Mattner *et al.* 2002) and which could not be studied without molecular techniques. Furthermore, genetic studies are more useful when complementary to field observations and the compound results provide more profound explanations and understandings of *why* and *how* things (population differentiation, natural selection,

reproductive isolation, allopathy, etc.) occur in such observed models (Orr & Smith, 1998; Piertney *et al.* 1998).

Although such integrated approaches are still at an infancy there is growing recognition, shown by recent research, of the potential role such approaches will have in the future (Orr & Smith, 1998; Robinson, 1999). Much recognition is felt and appreciated in the zoological field where the integrative approach is on the rise (Bernatchez *et al.* 1999; Robinson, 1999).

1.6 Problem Statement

Bush encroachment is a major concern in Africa and abroad and there are many species that cause bush encroachment. This phenomenon, through woody plants, affects the agricultural productivity and biodiversity of 10-20 million ha of South Africa (Ward, 2005). Many people believe that causes of bush encroachment are understood, but this seems not to be the case as we intimately engage with the problem. Other people believe that either heavy grazing by domestic livestock or fire is the sole cause of bush encroachment and this too appear not so definitive as bush encroachment does occur in many arid regions where fuel loads are insufficient for fires to be an important causal factor. Among the commonly recognized species capable of encroaching are *Acacia* species, which include *A. mellifera, A. karroo, A. reficiens,* and *A. tortilis* as well as *Dichrostachys cinerea*. Normally these encrouchers have thorns and secondary compounds (for instance phenolics) (Rohner & Ward 1997), which deter herbivores (Strauss *et al.* 2002). Following encroachment, these species (especially *Acacia mellifera*) form impenetrable thickets, thereby reducing the ability of the land to sustain people, livestock and game.

In South Africa, *A. mellifera* has been found to encroach and outgrow other plant species. The causes of the changes that have led to the present high densities of this plant in this semi-

arid savanna have been difficult to determine, and thus management of the species is currently a challenge. Two populations of *Acacia mellifera* were noted in Pniel, which is a semi-arid savanna, near Kimberley in the Northern Cape province of South Africa. One population appeared on a rocky, andesitic laval ridge, along the Vaal river whilst the other appeared sporadically in a sandveld area. Bush encroachment by *A. mellifera* was found to be more extensive on the rocky areas than in the sandveld. In particular, we would like to answer the following research questions**:**

1. Is the observed encroachment following any particular direction or is it completely random?
2. Is there a correlation between genetic and geographic distances amongst individuals, within a habitat type?
3. Are the two observed populations genetically different?
4. Could there be gene-by-environment (G x E) interaction yielding phenotypic plasticity?
5. Is there more genetic differentiation than local adaptation, which could imply two significantly different populations, which in turn would advocate for the two populations to be treated and managed differently?

These questions can be answered through molecular genetics, i.e. applying allozyme analyses to reveal and verify the level of genetic variability within and amongst populations. Furthermore, a completely-crossed design can be set up to test if whether or not there is local adaptation. Finally the following hypotheses can be tested

- **Genetic variability increases with geographic distance, i.e. individuals are genetically similar to their "mother" plant and less similar (genetically) to those**

further away. This hypothesis can help answer questions 2 and 3. In addition, by quantifying the number of allozymes detected in each cardinal direction could provide an indication of the probable direction bush encroachment is spreading in. Therefore, question 1 can also be answered.

- **There is no local adaptation in the two observed populations of *Acacia mellifera*, which were encroaching in Pniel rangelands.** This will help answer questions 4 and 5.

1.7 Aims & Objectives of the Study

My main aim was to determine the genetic differentiation, the direction of spread, and mode of reproduction of *A. mellifera* populations at Pniel in the Northern Cape, South Africa. Following the findings, means of control of encroachment by *A. mellifera* in semi-arid savannas will be recommended.

Preliminary observations of the study area showed that bush encroachment occurred mostly on andesitic laval rocky areas adjacent to the Vaal River, and secondarily in localized clusters in the adjoining sandveld. My main aim was to determine whether there was differentiation between and within *A. mellifera* sub-populations in these two habitat types and also to ascertain the direction of spread. I predicted that the *A. mellifera* plants are not introduced from great distances and consequently should have low genetic variability. Thus they would have increased in density or cover in this area because of changes in local abiotic or biotic conditions (Magnusson *et al.* 1999).

Due to the lack of knowledge of the population genetics of *A. mellifera* and the virtual absence of research on its population biology, little is known of its predominant mode of reproduction. *Acacia* species are known to exhibit both sexual and vegetative modes of reproduction (Davidson & Jeppe, 1981). Understanding the mode of reproduction will be useful in applying or devising a more appropriate technique (strategy) for curbing encroachment. For sexual reproduction, biological control measures can be applied and mechanical techniques will be resorted to if vegetative reproduction is identified. Consequently, I studied whether the observed encroachment by *A. mellifera* is accomplished through vegetative or sexual reproduction.

1.8 Thesis Structure

The thesis consists of an introduction chapter, a literature review chapter and two data chapters which are written in the forms of journal articles. The final part of the chapter is a synthesis chapter. The information covered in each of the chapters are as follows:

Chapter 1: This chapter serves to introduce the concept of bush-encroachment by providing a definition of savannas (a biome wherein bush-encroachment has extensively been observed) and attempting to quantify the spatial distribution of savannas, globally and also to provide a synopsis of the concept of local adaptation as a probable explanation for observed population differentiation in vegetation populations. Furthermore, contributions of employing molecular genetics in understanding population differentiation are looked at in this chapter. Finally the problem statement, the specific questions that all ushered in this particular research and the aims and objectives of the research study, are included in this chapter.

Chapter 2: In this chapter literature review around bush encroachment in savannas, especially by *Acacia mellifera* is summarized. The definition of savannas is explained further by looking at the explanations of tree:grass coexistence that have been brought forward and also the species that are currently known to encroach, are mentioned in this chapter. Factors that modify the tree:grass relationships are reviewed and findings in other similar research studies are quoted. The effects of bush encroachment in savannas, hydraulic lift and the subsequent negative impacts it has, not only in terms of biodiversity (flora & fauna) within a savanna, but also people's livelihoods are all reviewed. Finally, the chapter looks at probable causes of bush encroachment,

population conservation where population differentiation is evident and control measures to curb bush encroachment are suggested.

Chapter 3: This chapter looks at findings from electrophoresis based on seeds of *Acacia mellifera* with an attempt to quantify genetic similarity between and within two populations of *A. mellifera.* One population was observed on rocky, andesitic laval ridges of the study area (herein called the rocky population) whilst another population occurred in sandy soils and thus referred to as the sandveld population. The rocky population reflected extensive encroachment which covered vast areas in rocky habitats. On the sandveld, on the other hand, *A. mellifera* was observed encroaching rather differently as it appeared in clusters as opposed to a uniform spread noticed in the rocky areas. Population differentiation was suspected and as a result seeds were sampled from the two populations to conduct allozyme analysis in order to confirm the level of genetic similarity, mode of reproduction and the direction of spread. Knowing the direction of spread might be key in planning and controlling bush encroachment.

Chapter 4: A green-house experiment was conducted to test for local adaptation with the hypothesis that seeds collected from the rocky habitats would yield different seedlings when grown in the sandveld. Likewise, seeds from the sandveld when grown in rocky conditions, they would grow into different seedlings to those growing in the sandveld conditions. Therefore, a completely-crossed design was carried out and the study site conditions (rocky vs sandveld) were replicated using river sand, organic matter (cow-dung) and lime ($CaCO_3$). The sand used as medium was chosen on the grounds of pH as it had natural low pH and this made it easier to simulate the sandveld conditions

(low pH). Lime was then used to increase the pH to make it similar to the rocky habitat conditions. Finally, using the sand simplified experimentation because otherwise sand samples would have to be transported from the study area and this would bear high costs.

Chapter 5: In this chapter, I will draw an overall conclusion based on the findings of both data chapters, i.e. chapters 3 and chapter 4. Therefore this will be a synthesis for the entire research study.

1.9 LITERATURE CITED

Adams, M.E. 1967. A study of the ecology of *Acacia mellifera, A. seyal* and *Balanites aegyptiaca* in relation to land-clearing. *Journal of Applied Ecology* **4**: 221 – 273.

Asner, G.P, A.J. Elmore, L.P. Olander, R.E. Martin and A.T. Harris. 2004. Grazing systems, ecosystem responses, and global change. *Annual Review of Environment and Resources* **29**: 11.1 – 11.39

Baruch, Z. and B. Bilbao. 1999. Effects of fire and defoliation on the life history of native and invader C4 grasses in a Neotropical savanna. *Oecologia* **119**: 510 – 520.

Bernatchez, L., A. Chouinard and G. Lu. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus* sp., as a case study. *Biological Journal of the Linnean Society* **68**: 173 – 194.

Danthu, P., M. Ndongo, M. Diaou, O. Thuam, A. Sarr, B. Bedhiou and A.O. Mohamed Vall. 2003. Impact of bush fire on germination of some West African acacias. *Forest Ecology & Management* **107**: 1 – 10.

Davidson, L. and B. Jeppe. 1981. Acacias: A field guide to the identification of the species of Southern Africa. Centaur Publishers. Johannesburg. p. 6

de Jong, G. 1990. Genotype-by-environment interaction and the genetic covariance between environments: multilocus genetics. *Genetica* **81**: 171 – 177.

De Groot, R., J. van der Perk, A. Chiesura and A. van Vliet. 2003. Importance and threat as determining factors for criticality of natural capital. *Ecological Economics* **44**: 187 – 204.

Fenster, C.B., L.F. Galloway and C. Lin. 1997. Epistasis and its consequences for the evolution of natural populations. *Trends in Ecology & Evolution* **7**: 282 – 286.

Hernández-Hernández, R.M. and D, López-Hernández. 2002. Microbial biomass, mineral nitrogen and carbon content in savanna soil aggregates under conventional and no-tillage. *Soil Biology & Biochemistry* **34**: 1563 – 1570.

Huntley, B.J. 1982. Southern African savannas. *In.* Huntley B. J. and B.H. Walker. (eds). Ecology of tropical savannas. Springler verlag, Berlin. pp 101 – 119.

Huston, M.A. 1994. Biological diversity: the coexistance of species on changing landscapes. Cambridge University Press. Cambridge. pp 455 – 458.

Hutley, L.B. and S.A. Setterfield. 2008. Savanna. *Encyclopedia of Ecology*. Pp. 3143 – 3154.

Johnson, R.W. and J.C. Tothill. 1985. Definition and broad geographic outline of savanna lands. *In* Ecology and Management of the World's Savannas. J.C. Tothill & J.J. Mott (eds). Australian Academy of Science. Canberra. pp 1 – 13.

Magnusson, W.E., M. Carmozina-de-Araujo, R. Cintra, A.P. Lima, L.A. Martinelli, T.M. Sanaiotti, H.L. Vasconcelos and R.L. Victoria. 1999. Contributions of C3 and C4 plants to higher trophic levels in an Amazonian savanna. *Oecologia* **119**: 91 – 96.

Manlay, R. J., D. Masse, J. Chotte, C. Feller, M. Kairé, J. Fardoux and R. Pontanier. 2002. Carbon, nitrogen and phosphorus allocation in agro-ecosystems of a West African savanna; II. The soil component under semi-permanent cultivation. *Agriculture, Ecosystems & Environment* **88**: 233 – 248.

Mattner, J., G. Zwako, M. Rossetto, S.L. Krauss, K.W. Dixon and K. Sivasithamparam. 2002. Conservation genetics and implications for restoration of *Hemigenia exilis* (Lamiaceae), a serpentine endemic from Western Australia. *Biological Conservation* **107**: 37 – 45.

Moleele, N.M., S. Ringrose, W. Matheson and C. Vanderpost. 2002. More woody plants? The status of bush encroachment in Botswana's grazing areas. *Journal of Environmental Management* **64**: 3 – 11.

Orr, M.R. and T.B. Smith. 1998. Ecology and speciation. *Trends in Ecology & Evolution* **13**: 502 – 265.

Otter, L.B, A. Guenther and J. Greenberg. 2002. Seasonal and spatial variations in biogenic hydrocarbon emissions from southern African savannas and woodlands. *Atmospheric Environment* **36**: 4265 – 4275.

Piertney, S.B., A.D. MacColl, P.J. Bacon and J.F. Dallas. 1998. Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology* **7**: 1645 – 1654.

Radford, I.J., D.M. Nicholas and J.R. Brown. 2001. Impact of prescribed burning on *Acacia nilotica* seed banks and seedlings in the *Astrebla* grasslands of northern Australia. *Journal of Arid Environments* **49**: 795 – 807.

Radford, I.J., D.M. Nicholus & J.R. Brown. 2002. Biological control impact of seed predation on the invasive shrub *Acacia nilotica* (Prickly Acacia) in Australia. *Biological Control* **20**(3): 261 – 268.

Raven, P.H. and G.B. Johnson. 1992. Biology, 3rd ed. Mosby Year Book. USA. pp 389 – 390.

Ringrose, S., W. Matheson and C. Vanderpost. 1998. Analysis of soil organic carbon and vegetation cover trends along the Botswana Kalahari Transect. *Journal of Arid Environments* **38**: 379 – 396.

Robinson, G.E. 1999. Integrative animal behaviour and sociogenomics. *Trends in Ecology & Evolution* **14**: 202 – 205.

Rohner, C. and D. Ward. 1997. Chemical and mechanical defence against herbivory in two sympatric species of desert *Acacia Journal of Vegetation Science* **8**: 717 – 726.

Sankaran, M., Hanan, N.P., Scholes, R.J., Ratnam, J., Augustine, D.J., Cade, B.S., Gignoux, J., Higgins, S.I., Le Roux, X., Ludwig, F., Ardo, J., Banyikwa, F., Bronn, A., Bucini, G., Caylor, K.K., Coughenour, M.B., Diouf, A., Ekaya, W., Feral, C.J., February,

E.C., Frost, P.G.H., Hiernaux, P., Hrabar, H., Metzger, K.L., Prins, H.H.T., Ringrose, S., Sea, W., Tews, J., Worden, J., Zambatis, N. 2005. Determinants of woody cover in African savannas. *Nature* **438**: 846-849.

Schlichting, C.D. 1986. The evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **17**: 667 – 693.

Scholes, R.J. and B.H. Walker. 1993. An African Savanna: Synthesis of the Nylsvley Study. Cambridge. Cambridge University Press. pp 190 – 207.

Scholes, R.J. 1997. Savanna. *In* Cowling, R.M., D.M. Richardson and S.M. Pierce (eds). Vegetation of Southern Africa. Cambridge University Press. United Kingdom. pp 258 – 277.

Scholes, R.J., P.R. Dowty, K. Caylor, D.A.B. Parsons, P.G.H. Frost & H.H. Shugart. (2002). Trends in savanna structure and composition along an aridity gradient in the Kalahari. *Journal of Vegetation Science* **13**: 419 – 428.

Shrestha, M.K., A. Golan-Goldhirsh and D. Ward. 2002. Population genetic structure and the conservation +of isolated populations of *Acacia raddiana* in the Negev Desert. *Biological Conservation* **108**: 119 – 127.

Smit, G.N. 2004. An approach to tree thinning to structure southern African savannas for long-term restoration from bush encroachment. *Journal of Environmental Management* **71**(2): 179 – 191.

Strauss S.Y., J.A. Rudgers, J.A. Lau & Irwin RE. 2002. Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution* **17**: 278 – 284.

Taberlet, P. and G. Luikart. 1999. Non-invasive genetic sampling and individual identification. *Biological Journal of the Linnean Society* **68**: 41 – 55.

Teague, W.R. and G.N. Smit. 1992. Relations between woody and herbaceous components and the effects of bush-clearing in southern African savannas. *Journal of the Grasslands Society of southern Africa* **9**: 60 – 69.

Van Auken, O.W. 2009. Causes and consequences of woody plant encroachment into western North American grasslands. *Journal of Environmental Management* **90**(10): 2931 – 2942

van de Vijver, C.A.D.M., C.A. Foley and H. Olff. 1999. Changes in the woody component of an East African savanna during 27 years. *Journal of Tropical Ecology* **15:** 545 – 564.

Vitousek, P.M. 1992. Global environmental change: An introduction. *Annual Review of Ecology and Systematics* **23**: 1 – 14.

Walter, H. 1939. Grassland, savanne, und busch der ariden teile Afrikans in ihrer ökologischen. *Bedingtheit Jaarboek Wissenschaftliche Botanie* **87**: 750 – 860.

Ward, D. 2005. Do we understand the causes of bush encroachment in African savannas? *African Journal of Range & Forage Science* **22**(2): 101 – 105.

Chapter 2

LITERATURE REVIEW

2.1 Determinants of Savanna Structure

Africa is covered by vast areas of savanna. These have a structural variation ranging from a few scattered trees in grasslands in low rainfall areas to high rainfall areas, which mostly have woody trees and a low density of grass. This variation is a result of, and can be influenced by, many factors such as rainfall, fires and overgrazing (Belsky, 1992; Olff & Ritchie, 1998; Biggs *et al.* 2002; Sankaran *et al.* 2004; Savadogo *et al.* 2009). Many theories aimed at explaining the tree-grass coexistence of savannas, have been put forward including the classic paradigm that grasses have shallow root systems that can utilize topsoil nutrients and water (Walter, 1939; Huston, 1994) and thus outcompete the woody plants (which have deep penetrating roots and are slow growing). All of these have fallen short of providing a globally accepted generalization regarding the savanna vegetation dynamics.

According to Ludwig *et al.* (2004) and Sankaran *et al.* (2004), even the emerging consensus on niche partitioning may not be sufficient to explain tree-grass coexistence in savannas. Higgins *et al.* (2000) suggested an alternative theory that recognizes the role of fire and resprouting ability of trees in determining tree:grass relationships. Other contributors may include soil erosion (Badejo, 1998) and overgrazing (Belsky, 1992; Olff & Ritchie, 1998; Biggs *et al.* 2002), which allows grasses to be removed and the remaining woody plant seeds would have less competition (Van Auken, 2000). Their seeds make productive use of the unutilized topsoil nutrients and water, because woody plant seeds need more water for imbibition (Testerink *et al.* 1999) than do grass seeds.

A recent (local) study by Kraaij and Ward (2006), showed that an interaction between many factors might shed some light on savanna dynamics, functioning and bush encroachment; these factors are precipitation, soil nutrients, fire and herbivory (also see

Schulz *et al.* 1955; Frost *et al.* 1985; Knoop & Walker, 1985; Van Auken *et al.* 1985; Walker & Knoop, 1987; Stuart-Hill & Tainton, 1988, 1989; Teague & Smit, 1992; Jeltsch *et al.* 1996; Higgins *et al.* 2000; Savadogo *et al.* 2009). Apparently, the interaction between these factors determine the tree:grass ratio and eventually the occurrence/absence of bush encroachment (Sankaran *et al.* 2004).

In the absence of competition from grasses, trees germinate and take over the available land. Thus disturbances within savannas can modify tree:grass relationships and lead to increased woody cover (often unpalatable to livestock) at the expense of (palatable) grass cover and resources, which is termed bush encroachment (Van Auken, 2000). The commonly recognized woody species implicated in bush encroachment are some *Acacia* species, viz. *A. mellifera, A. karroo, A. reficiens, A. tortilis* and *Dichrostachys cinerea*, which have thorns and secondary compounds (for instance phenolics) which deter herbivores (Rohner & Ward, 1997; Adler, 2000). Because trees require more rain to germinate than do grasses and may even germinate *en masse* with or without grazing in rare, high rainfall years (Ward & Rohner, 1997), it is proposed that rainfall amount and frequency might have an important role in the occurrence of bush encroachment.

Another study was conducted in southern Ethiopia (Oba *et al.* 2000) to assess the relationships between bush cover, grass cover and bare soil and grazing pressure and soil erosion and changes in range condition, in dry savannas. In this study, bush cover was found to be negatively correlated with grass cover and positively correlated with bare soil. Grass cover was negatively correlated with bare soil and grazing pressure in most landscape patch types. Grazing pressure was not significantly correlated with bush cover or bare soil, while soil erosion was directly related to bare soil. Therefore, factors that lead to a decrease in the density of grasses (Badejo, 1998) promote the growth of bushes, although the availability of bare ground does not lead to an increase in bush cover.

Most likely, grasses function to enhance the sedimentation of nutrients (Walter, 1939; Badejo, 1998), as opposed to bare ground, where there is surface runoff (Bastian & Roeder, 1998). Grasses may indirectly increase both the fertility and aeration of the soil (Khan, 1999), infiltration of water into the soil, which may all be conducive for woody plant seeds to germinate.

2.2 Savannas and Bush-encroachment

Savannas are biomes most widespread in the tropics and as such are subjected to human impacts because of anthropogenic activities associated with the increasing population growth (Peres, 1998; Shackleton, 2000). They are largely constituted of trees and grasses (Liedloff *et al.* 2001), which are normally the dominant life forms. In spite of their spatial extent and importance as a biome, the origin, age, nature and dynamics are still not yet well understood (Scholes, 1997). The main question about savannas revolves around the long-term co-existence of the dominant life forms as to how they co-exist without one outcompeting the other, what mechanisms determine the proportion of each and how do they persist as savannas when the equilibrium state is disturbed? (Jeltsch *et al.* 1996; Scholes, 1997; Folster *et al.* 2001; Liedloff *et al.* 2001; Laclau *et al.* 2002). The disturbance of savanna equilibrium results in one of the life forms dominating the other. That is, the savanna changes either to pure grasslands or forests and the gradual change from an open savanna to closed savanna is termed bush encroachment.

In southern African savannas, bush encroachment has proved to be a major problem for range managers (Dahlberg, 2000). Following bush-encroachment, the observed structural variation that exists in southern African savannas is altered. The savanna structure is composed of layers whereby C_4 grass cover potentially dominate both the herbaceous and the woody strata; these grasses are usually fire-dominant and have a density variation ranging

from a sparsely dense to a closed woodland (Baruch & Bilbao 1999; Magnusson *et al.* 1999). The former constitutes open savannas and the latter, closed savannas. Rainfall occurs in the warmer, summer months with a dry period of between two to eight month's duration during which fire is a typical phenomenon (Huntley, 1982).

In the Kalahari Desert of Botswana, as in many other open savannas, the main ecological change following cattle-based agricultural intensification is one of grass removal and bush encroachment (Meik *et al.* 2002; Moleele *et al.* 2002). Changes in vegetation communities in Kalahari rangelands have been expressed in terms of a state-and-transition model (Dahlberg, 2000). However, there remain uncertainties as to the mechanisms and conditions for ecological change. It is the lack of such knowledge and the incompleteness of available information concerning the effects of herbivores on herbaceous vegetation and primary productivity which worsens the situation, especially if land-users (for instance farmers) do not know or are not aware of the factors that disturb the savanna equilibrium. However, in Pniel (my study area), it was found that heavy grazing reduces fuel loads and consequently less frequent and intense fires, further reducing the effectiveness of fire in controlling woody vegetation (Britz & Ward, 2007). Furthermore, heavy grazing alters competitive interactions between woody and herbaceous layers through the removal of grasses (Skarpe, 1990; Hoffman & Ashwell, 2001).

About 20 million hectares of South African lands are currently influenced by bush encroachment (Ward, 2005). It is the combination of thorns and low digestibility, due to the presence of secondary compounds, of *Acacia* trees that reduces their accessibility and nutritional value to livestock (Midgley & Ward, 1996, Rohner & Ward, 1997). This reduces the ability of the land to support livestock and indirectly, people (Marchant, 2010). Bush encroachment can convert vast areas of land into less productive land forms for many years (40-60yrs) (Badejo, 1998). This may last until competition for nutrients between the trees,

fire and other causes of mortality occur to reduce tree density and once again allow for grass regrowth (Holdo, 2007; Savadogo *et al.* 2009). Thus, bush encroachment can lead to serious resource constraints for livestock and human.

Because savanna ecosystems are usually vulnerable to transitions from grasslands to shrublands through woody plant encroachment, these transitions result in potentially significant shifts in the functions of such ecosystems (Badejo, 1998; Hudak & Wessman, 1998). Furthermore, they pose problems to range managers (Witkowski & Garner, 2000) as it results in habitat fragmentation and subsequent declines in territorial grassland species (Helzer & Jelinski, 1999; Walk & Warner, 1999; Winter & Faaborg, 1999; O' Leary & Nyberg, 2000; Kraaij & Ward, 2006; Britz & Ward, 2007).

2.3 Causes of Bush Encroachment by Bush-encroaching Species

Bush encroachment (bush thickening, or thicket formation as it is also referred to) is a serious vegetation concern, not only affecting savanna biomes. Four environmental variables are recognized as significantly influencing woody plant species composition along the grazing gradients, viz., cattle density, soil nitrogen, distance from foci points and tree cover (Moleele & Perkins, 1998). Out of these four variables, cattle density was found to explain about 33% of the variance out of the total 60% explained by the four variables, in Botswana. Bush encroachment is known to cause significant reductions in rangeland quality, reduce the ability of the land to support both people and their livestock, affect biodiversity and might also alter the soil-water structure (Moleele *et al.* 2002; Marchant, 2010).

In addition, factors that promote bush encroachment are not always easy to identify and this exerts much pressure on curbing the problem, whose effect is unquestionable. Moleele *et al*. (2002) fully acknowledges that, even though some work on the extent of woody cover and the causes of bush encroachment is being undertaken, conducting more

research is of high importance. So doing will help get more information and obtain more specific, practical results. Among the commonly recognized species capable of encroaching are legume species such as *Acacia* species, which include *A. mellifera, A. karroo, A. reficiens,* and *A. tortilis* as well as *Dichrostachys cinerea*. These normally have thorns and secondary compounds (for instance phenolics) (Rohner & Ward, 1997; Adler, 2000), which deter herbivores (Strauss *et al.* 2002). Following encroachment, these species (especially *Acacia mellifera*) form impenetrable thickets, thereby reducing the ability of the land to sustain both people and their livestock (Marchant, 2010). Bush encroachment is widespread and affects land owned by both black and white farmers, in spite of the differing socioeconomic, cultural and political forces (Hudak, 1998).

Research has shown that trees require more rain to germinate than do grasses and may even germinate *en masse* with or without grazing in rare, high rainfall years (Ward & Rohner, 1997; Garcia & Jurado, 2003). Soil type might also be supportive of encroachment, depending on its water retention capacity (Dahlberg, 2000). Although comprehensive information on water relations and soil water uptake patterns is still lacking, it is shown that soils with higher water retention capacity (e.g. more clayey soils) sustain woody plant growth (Mackay, 2001). Such soils play an important role in variations in vegetation physiological activity, plant phenology and potential competitive interactions between dominant life forms (Mackay, 2001) as encroachment is shown to increase with soil clay content (Britz & Ward, 2007). It is therefore predicted that rainfall amount and frequency might have an important role in the prevalence of bush encroachment and to a certain extent, this would suggest that farmers should not overstock during wet years but rather to employ other land management options.

In addition, the seed germinability under certain environmental conditions might be a contributing factor (Kraaij & Ward, 2006). Scarified seeds of *Dichrostachys cinerea* have

been reported to germinate better under the interaction of light and availability of disturbed soils (Vilela & Ravetta, 2001). Scarification might also be improved by herbivores eating the seeds and subsequent to passage through the gut, the seeds are scarified and have a higher germinability (Vilela & Ravetta, 2001). Their presence in the dung also provides them with a conducive microhabitat in which to germinate (Vilela & Ravetta, 2001).

Other evidence of the effect of encroaching species, through bush encroachment was shown by Hudak (1998), in his study at Madikwe Game Reserve, South Africa. Here, chronic, heavy livestock grazing and concomitant fire suppression were reported to have caused the gradual replacement of palatable grass species by less palatable woody species. Policymakers and cattle farmers alike have not appreciated the ecological role fire and native browsers, under appropriate burning regimes and stocking management, play in preventing bush encroachment. Unpredictable droughts are common in South Africa but have deflected too much blame for bush encroachment away from grazing mismanagement (Hudak, 1998). The gradual conversion of grassy, open savannas into woody, closed savannas, which are not as supportive to both humans and their livestock as the open savannas, is easily attributed to the observed droughts.

2.4 **Acacia mellifera** *and its Role in Bush-encroachment*

The agricultural importance of *Acacia mellifera*, in spite of being an edible shrub, is still not yet recognized. However research on N-fixation, by leguminous species of which *A. mellifera* is a member of, has shown that grain legumes can fix about 15–210 kg N ha^{-1} annually in Africa, and thus feature prominently in the cropping systems of traditional farmers and this makes the foliage of these legumes the "ideal" fertilizer (Dakora & Keya, 1997). Tree legumes were quantified to fix about 43–581 kg N ha^{-1} y^{-1}, making their leaf prunings an even more important component of sustainability in agroforestry and alley cropping systems (Dakora & Keya, 1997). Other research has shown that foliage from tree

legumes given as supplement to a diet of maize for livestock, e.g. sheep, can improve dry matter intake, digestibility of organic matter, nitrogen balance and microbial protein yield (Masama *et al.* 1997). In addition, although browsed by goats to a certain extent, it still cannot be regarded as fodder because of its herbivore defence mechanism (van Wyk & van Wyk, 1987; Strauss *et al.* 2002; Dziba *et al.* 2003). *Acacia mellifera* is one known acacia species which invests a great deal in secondary metabolism (phenolic compounds) which deter browsers (Barroso *et al.* 2001). In addition, since it is thorny its leaves are not easily accessible. However, in some cases *A. mellifera* is used as a nitrogen-fixing plant (since nodulation is observed and known for nitrogen fixation), for shade provision and as a fence plant (hedge), around houses (Carr, 1976; Coe & Beentje, 1991).

Acacia mellifera is a shrub or small tree, commonly known as Black thorn in English, Swarthaak, in Afrikaans (van Wyk & van Wyk, 1987; Davidson & Jeppe, 1981), and belongs within the family Mimosaceae. It is a multi-stemmed shrub that can grow up to 3 m high, has flat crowns and is very thorny, forming impenetrable thickets, especially in overgrazed areas (van Wyk & van Wyk, 1987). *Acacia mellifera* is found in bushveld and semi-desert areas, often on Kalahari sands (van Wyk & van Wyk, 1987). It also spreads from the central northern parts of South Africa (including the Northern Cape), up through northwest Namibia, through Botswana into Zimbabwe. Some are also found in Zambia, and in the Zimbabwe and Mozambique border (van Wyk & van Wyk, 1987; Davidson & Jeppe, 1981).

The plant is browsed by stock (van Wyk & van Wyk, 1987), especially goats and camels. However, it is considered too spiny for cattle to browse (Biggs *et al.* 2002), during rainy seasons when the leaves are fleshy. Game, gerenuk and other ruminants (Wickens *et al.* 1995), also browse it. In addition, it also is a good nectar source for bees (Palgrave, 1977). Subsequent to responding to changes in certain factors such as rainfall, herbivory, fire and soil nutrients, *A. mellifera* is well known to encroach. Bush encroachment is suspected, not

only to threaten biodiversity but also to alter the soil-water structure through hydraulic lift (Moleele *et al.* 2002; Porporato *et al.* 2002). In the study site, Pniel, bush encroachment is relatively prominent and most interestingly, there appeared to be two populations of *A. mellifera* which, hypothetically, seem to be encroaching the area. In spite of encroachment being so disadvantageous, should the two populations be a reality, it could be ecologically detrimental to exterminate one or both of the populations.

Collecting information and improving an understanding of how the species reacts to certain environmental factors after which the species begins to encroach and also how it reacts to variable levels of herbivory can assist land managers and scientists gain an insight into the species' population genetics (Savadogo *et al.* 2008; Marchant, 2010). Also of great importance in understanding the species is how it responds to local environmental conditions such as soil texture, i.e. variable levels of clay and silt in the soil, soil moisture content, and rockiness of the habitat. The species is suspected to be able to propagate through both sexual reproduction and vegetative reproduction and local environmental conditions might be the required trigger to cause the species to switch between reproduction modes. All this information is crucial in understanding savannas and savannas deserve a special place on the ecosystem management agendas in order to ensure a sustainable future for savanna role players, including biodiversity and humans. As such it becomes vital that knowledge gaps in ecological function, natural resource partitioning and predicted response of savannas to environmental change are filled (Marchant, 2010)

Due to the lack of scientific data especially on population genetics, on *A. mellifera*, little information is known about the mode of encroachment, factor(s) that induce encroachment and the gradient of distribution of this *Acacia* species. Bush encroachment of *A. mellifera* has been reported in Kimberley (Northern Cape, South Africa), on communal and commercial farms called Pniel Estates, which is the study site for this research (Kraaij &

Ward, 2006; Britz & Ward, 2007). This farm is mainly savanna, with an annual rainfall of between 400 and 450mm. Due to the encroachment of *A. mellifera*, the area has changed from open savanna into dense thickets of *A. mellifera*, which are not edible, thereby reducing grazing spaces for livestock. Two modes of encroachment have been predicted and were tested in this project, viz. (1) vegetative propagation and (2) seed dispersal. In order to ascertain which mode of reproduction *A. mellifera* is actually propagating by, isozyme analyses was carried out. Allozyme analysis is just one of the means through which curbing bush-encroachment, which has been unsuccessful so far (Teague & Smit, 1992), might be achieved. This failure to contain bush encroachment may be direct evidence and consequence of the lack of knowledge of the ecological mechanism(s) that causes bush encroachment.

2.5 Consequences of Bush-encroachment for Populations of Plants and Animals

Following the observations of two populations of *Acacia mellifera*, in the study area, if population differentiation could be established in which case the implications would be that the two populations are now different, conservation of each population would be recommended. From a conservation point of view, according to Shrestha *et al.* (2002), population conservation is highly important because, as populations are genetically differentiated, population loss leads to a dramatic depletion in genetic variation. Shrestha *et al.* (2002), further acknowledges the significance of genetically distinct populations that they yield out-breeding depression, because of GXE (gene by environment) interactions. However, the implication of this significance is that different populations might require different management plans. Therefore, where encroachment is observed and population differentiation is also prominent, it might be recommended that any applied control treatments not threaten any of the populations but merely limit the effect.

Within a limited area, where there is coexistence of different plant communities and sustainable, equal sharing of resources, the impacts of encroachment can be visualized as, spatial dominance, reduction in biodiversity and possibly, habitat rearrangement. Spatial dominance might result in competition for resources (soil nutrients, light and water) which would out-compete the less competitive species, for a particular resource (Curtin *et al.* 2002). This then results in plant succession where in the case of woody plants dominating the herbaceous layer and the overall local biodiversity drops with an increase in woodies (Milton *et al.* 1994; Angassa & Oba, 2010). The shade produced by canopy of trees, rapidly begins to selectively allow herbaceous species that can tolerate shade or stunt and even kill other herbaceous species and alters the grass-dominated ground cover (Tews *et al*, 2006; Angassa & Oba, 2010). This shaded area eventually becomes colonized by shade-loving shrubs and this further creates an ideal habitat for other tree species to establish themselves (Tews *et al.* 2006). As the herbaceous layer, especially grass biomass, density and cover declines the density of woody invasive species increases and biodiversity is compromised (Tews *et al.* 2006; van Auken, 2009; Angassa & Oba, 2010). Even the overall biomass of the savanna is gradually shifted from below ground, in the case of grassland, to above-ground in the encroached state of the savanna (van Akuen, 2009). As a result the ability of the savanna to support game, livestock (grazing capacity) or even to benefit land-users, such as farmers, also declines as both species richness and biodiversity change when woody species replace the herbaceous layer (Smit, 2004; Tews *et al.* 2006; van Auken, 2009).

The impacts of encroachment on fauna are gradually being recognized (Twyman, 2001). In the absence of encroachment (under normal conditions), faunal diversity is usually reasonably high in African savannas; there might be different herbivore species dependant on the floral community, which is also reasonably diverse. However, in the presence of encroachment and with the effect of competition, which excludes the less competitive flora

species, both species richness and species diversity (fauna) might also be threatened (El Aich & Waterhouse, 1999; Meik *et al.* 2002). In such a case, only species that are favoured by the encroaching species might be found in abundance (Moleele *et al.* 2002). In a research conducted in Botswana, it was found that thornveld species tend to benefit from this vegetation change (Herremans, 1998). From this, it can be deduced that grassland birds and some birds of prey get negatively affected, whereas those of canopy woodland luxuriate (Herremans, 1998; Thiele *et al.* 2008).

Therefore, bush encroachment changes the spatial structure of the savannas, the tree-grass density and may thus rearrange the ecosystem. This rearrangement might impact on the savanna fauna, rendering bush encroachment both an economic and environmental hazard (Moleele *et al.* 2002). In a survey on diurnal lizard assemblages conducted on Namibian rangelands, it was found that the decreased habitat diversity associated with encroachment influences native savanna lizard assemblages (Meik *et al.* 2002).

2.6 Consequences of Bush-encroachment for Soil Physical and Chemical Properties

Heavy encroachment by woody species, which have long and deep penetrating root systems, is now being suspected to be responsible for hydraulic lift whereby the water table is lifted and brought closer to the surface thus benefiting a range of woody plants and some herbaceous species (Porporato *et al.* 2002). Although not much research in this regard has been conducted on *A. mellifera*, evidence shows that soil-water becomes shallow where there are many woody plants or thinning and this was evident and attributed to tree thinning in the Limpopo province semi-arid savanna (Smit & Rethman, 2000). Here, the soil-water was held at a shallow depth of less than 450mm, whereas in the non-encroached areas it was found between 450 and 900mm, depending on the rainfall event. On the contrary, mean evapotranspiration was found markedly higher in non-encroached areas than in the

encroached ones. This observation was attributed to the fact that grasses utilize soil-water more rapidly than the woody trees (Smit & Rethman, 2000).

Furthermore, trees in savannas have been found to modify soil nutrient conditions for grasses but whether this has an impact on the quality of herbaceous vegetation is still not clear. The effect of savanna trees on soil nutrient condition was found evident in a study conducted in eastern and southern Africa where sub-canopy (SC) grasses were found to be significantly different to grasses outside tree canopies, in terms of structure and nutrient levels (Treydte *et al.* 2007; Treydte *et al.* 2009). The study concluded that trees, especially in dry savannas, improve grass quality and could attract grazing ungulates and as such woody plant clearing should be limited in low fertility savannas and their benefits for grazing wildlife be recognized in conservation strategies (Treydte *et al.* 2007; Treydte *et al.* 2009). However, this should be seen within the suppressive effect bush encroachment has on grass cover such that gains in fertility comes at the expense of grass cover, as noted above.

2.7 Combating Bush-encroachment, specifically by **Acacia mellifera**

Coming up with an effective treatment for controlling bush encroachment by *A. mellifera*, will largely depend on what the cause is. Should heavy grazing, as suspected, be the cause then enforced reduction of livestock from grassland areas, which are still susceptible to encroachment, might be recommended (Moleele *et al.* 2002). It also has been recognized that livestock (cattle) husbandry and the shifting of focal points (water-points and kraals) could be factors encouraging the germination and survival of encroachers (Moleele *et al.* 2002). To a certain degree, plagues of grasshoppers can remove young grasses, which if happening at the beginning of a rainy season, can cause the same results as overgrazing (Adams, 1967).

Furthermore, knowing the mode of reproduction by which the encroaching species reproduce might have an important role in recommending a control treatment. For instance, if

sexual reproduction is observed, introducing exotic, seed-infesting insects such as bruchid beetles (coupled with cattle removal) or applying any other bio-control, might be beneficial (Radford *et al.* 2001; Rohner & Ward, 1997). However, if there is vegetative reproduction, mechanical means like uprooting the seedlings when they are still young might be recommended. Otherwise, if encroached areas are left unattended, the grass-woody plant dynamics which identifies stable savannas, might never be reached until the encroaching *Acacia* eventually die out (Boutton *et al.* 1998). Once the old bushes die out, then grasses re-enter the system and if fire is introduced, it kills the *Acacia* stands and the system may return back to a grass dominated savanna. Adams, (1967) acknowledges the change from thicket to grassland might be straightforward but the reverse transition from grassland to thicket is not so obvious.

On areas that are already encroached, dealing with the problem might not be as easy as acacias are known to have secondary metabolites, phenolics, which deter herbivores (Wendel & Weeden, 1989). However, Erasmus (2000) recommends an introduction of Boer goats in such areas. He reckons goats are notably effective in combating undesirable encroachment. The approach to combating bush encroachment, however, needs an understanding of several basic genetic and ecological issues. Of these, the existence of local adaptation, as well as the mode of reproduction is critical to tailor a method for removing encroachers. This thesis is designed to answer two of these critical questions, namely whether local adaptation exist in *A. mellifera* populations observed in the study area, and what the mode of reproduction was.

Much of the modifications in the savanna dynamics can then be accredited to the widespread effects of intensive grazing by livestock, which results in a reduction of the herbaceous stratum and eventually in bush encroachment. (Herremans, 1998). In an attempt to curb bush encroachment, managers at Madikwe Game Reserve have reintroduced fire and

native game animals into a formerly overgrazed system, with encouraging preliminary results (Hudak, 1998). Of paramount need is a bush control program that educates cattle farmers about the ecological causes of bush encroachment, informs policies and encourages the use of fire and native browsers as tools for sustainable grazing management (Hudak, 1998; Eriksen & Watson, 2009). It thus becomes a necessity that land users and managers should be well equipped with physiological and genetic knowledge on savannas, as well as the soil water and vegetation dynamics through which these biodiversity-rich biomes maintain their identity.

For long-term purposes, educating land-users and managers could benefit the ecosystem, accommodate the needs and also fulfil the expectations of the land-users and in this way, the biome will also be assisted in its persistence into the future. Identifying the problems and attending to them, without regarding farmers and managers as part of the whole system may only serve as short-term goals. The profound and empirical solutions to the problems that threaten the theoretical character of these biomes might be used as a foundation upon which to set policy rules, be conserved as basis of improvement and be passed on from generation to generation.

2.8 LITERATURE CITED

Adams, M.E. 1967. A study of the ecology of *Acacia mellifera, A. seyal* and *Balanites aegyptiaca* in relation to land-clearing. *Journal of Applied Ecology* **4**: 221 – 273.

Adler, L.S. 2000. Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. *American Naturalist* **156**: 92 – 99.

Angassa, A. & G. Oba. 2010. Effects of grazing pressure, age of enclosures and seasonality of bush cover dynamics and vegetation composition in southern Ethiopia. *Journal of Arid Environments* **74**(1): 111 – 120.

Badejo, M.A. 1998. Agroecological restoration of savanna ecosystems. Ecological Summit 96, Copenhagen. Denmark. **10**: 209 – 219.

Barroso, F.G.,T.F. Martinez, T. Paz, A. Parra, & F.J. Alarcon. 2001. Tannin content of grazing plants of southern Spanish arid lands. *Journal of Arid Environments* **49**: 301 – 314.

Baruch, Z. and B. Bilbao. 1999. Effects of fire and defoliation on the life history of native and invader C4 grasses in a Neotropical savanna. *Oecologia* **119**: 510 – 520.

Bastian, O. & M. Roeder. 1998. Assessment of landscape change by land evaluation of past and present situation. *Landscape and Urban Planning* **41**: 171 – 182.

Belsky, A.J. 1992. Effects of grazing, competition, disturbance and fire on species composition and diversity in grassland communities. *Journal of Vegetative Science* **3**: 187 – 200.

Biggs, T.H., J. Quade & R.H. Webb. 2002. ^{13}C values of soil organic matter in semi-arid grassland with mesquite (*Prosopis*) encroachment in southern Arizona. *Geoderma* **110**: 109 – 130.

Boutton, T.W, S.R. Archer, A.J. Midwood, S.F. Zitzer & R. Bol. 1998. δ^{13}C values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. *Geoderma* **82**: 5 – 41.

Britz, M-L & D. Ward. 2007. Dynamics of woody vegetation in a semi-arid savanna, with a focus on bush encroachment. *African Journal of Range & Forage Science* **24**(3): 131 – 140.

Carr, J.D. 1976. The South African acacias. Conservation Press. Johannesburg. pp 243 – 245.

Coe, M. & H. Beentje. 1991. A field guide to the Acacias of Kenya. Oxford University Press. New York. pp 17, 98 – 99.

Curtin, C.G., N.F. Sayre, & B.D. Lane. 2002. Transformation of the Chihuahuan Borderlands: grazing, fragmentation, and biodiversity conservation in desert grasslands. *Environmental Science & Policy* **5**: 55 – 68.

Dahlberg, A.C. 2000. Vegetation diversity and change in relation to land use, soil and rainfall — a case study from North-East District, Botswana. *Journal of Arid Environments* **44**: 19 – 40.

Dakora, F.D. & S.O. Keya. 1997. Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. *Soil Biology and Biochemistry* **29**(5-6): 809 – 817.

Davidson, L. & B. Jeppe. 1981. Acacias: A field guide to the identification of the species of Southern Africa. Centaur Publishers. Johannesburg. p. 6.

Dziba, L.E., P.F. Scogings, L.J. Gordon and J.G. Raats. 2003. Effects of season and breed on browse species intake rates and diet selection by goats in the False Thornveld of the Eastern Cape, South Africa. *Small Ruminant Research* **47**: 17 – 30.

El Aich, A. & A. Waterhouse. 1999. Small ruminants in environmental conservation. *Small Ruminant Research* **34**: 271 – 287.

Erasmus, J. A. 2000. Adaptation to various environments and resistance to disease of the improved Boer goat. *Small Ruminant Research* **36**: 179 – 187.

Eriksen, S.E.H. & H.K. Watson. 2009. The dynamic context of southern African savannas: investigating emerging threats and opportunities to sustainability. *Environmental Science & Policy* **12b**(1): 5 – 22.

Folster, H., N. Dezzeo and J.A. Priess. 2001. Soil–vegetation relationship in base -deficient premontane moist forest–savanna mosaics of the Venezuelan Guayana. *Geoderma* **104**: 95 – 113.

Frost, P., E. Medina, J.C. Menaut, O. Solbrig, M. Swift and B. Walker (eds). 1985. Responses of savanna to stress and disturbance. In: *Biological International* **10**: 1 – 82.

Garcia, J.F. & E. Jurado. 2003. Influence of plant cover on germination in matorral in northeastern Mexico. *Forest Ecology & Management* **177**: 11 – 16.

Helzer, C.J. & D.E. Jelinski. 1999. The relative importance of patch area and perimeter-area ratio to grassland breeding birds. *Ecological Applications* **9**: 1448 – 1458.

Herremans, M. 1998. Conservation status of birds in Botswana in relation to land use. *Biological Conservation* **86**: 139 – 160.

Higgins, S.I., W.J. Bond and W.S.W Trollope. 2000. Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. *Journal of Ecology* **88**: 213 – 229.

Hoffman, T & A. Ashwell. 2001. Nature divided: Land degradation in South Africa. University of Cape Town Press, Cape Town, South Africa.

Holdo, R.M. 2007. Elephants, fire and frost can determine community structure and composition in Kalahari woodlands. *Ecological Applications* **17**: 558 – 568.

Hudak, A.T. & C.A. Wessman. 1998. Textural analysis of historical aerial photography to characterize woody plant encroachment in South African savanna. *Remote Sensing of Environment* **66**: 312 – 330.

Huntley, B.J. 1982. Southern African savannas. *In.* Huntley B.J. and B.H. Walker. (eds). Ecology of tropical savannas. Springler verlag, Berlin. pp 101 – 119.

Huston, M.A. 1994. Biological diversity: the coexistance of species on changing landscapes. Cambridge University Press. Cambridge. pp 455 – 458.

Jeltsch, F., S.J. Milton, W.R.J. Dean and N. Van Rooyen. 1996. Tree spacing and coexistence in semi-arid savannas. *Journal of Ecology* **84**: 583 – 959.

Khan, M. A. 1999. The hydrological response and nutrient loss of certain horticultural ecosystems. *Journal-of-Arid-Environments* **42**: 281 – 289.

Knoop, W.T. & B.H. Walker. 1985. Interactions of woody and herbaceous vegetation in a southern African savanna. *Journal of Ecology* **73**: 235 – 253.

Kraaij, T. & D. Ward. 2006. Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology* **186**: 235 – 246.

Laclau, J., W. Sama-Poumba, J. Nzila, J. Bouillet & J. Ranger. 2002. Biomass and nutrient dynamics in a littoral savanna subjected to annual fires in Congo. *Acta Oecologica* **23**: 41 – 50.

Liedloff, A.C., M.B. Coughenour, J.A. Ludwig & R. Dyer. 2001. Modelling the trade -off between fire and grazing in a tropical savanna landscape, northern Australia. *Environment International* **27**: 173 – 180.

Ludwig, F., T.E. Dawson, H.H.T. Prins, F. Berendse & H. De Kroon. 2004. Below-ground competition between trees and grasses may overwhelm the facilitative factors of hydraulic lift. *Ecology Letters* **7**: 625 – 631.

Mackay, D.S. 2001. Evaluation of hydrologic equilibrium in a mountainous watershed: incorporating forest canopy spatial adjustment to soil biogeochemical processes. *Advances in Water Research* **24**: 1211 – 1227.

Magnusson, W.E., M. Carmozina-de-Araujo, R. Cintra, A.P. Lima, L.A. Martinelli, T.M. Sanaiotti, H.L. Vasconcelos and R.L. Victoria. 1999. Contributions of C3 and C4 plants to higher trophic levels in an Amazonian savanna. *Oecologia* **119**: 91 – 96.

Marchant, R. 2010. Understanding complexity in savannas: climate, biodiversity and people. *Current Opinion in Environmental Sustainability* **2**(1-2): 101 – 108.

Masama, E., J.H. Topps, N.T. Ngongoni & B.V. Maasdorp. 1997. Effects of supplementation with foliage from the tree-legumes *Acacia angustissima, Cajanus cajan, Calliandra calothyrsus* and *Leucaena leucocephala* on feed intake, digestibility and nitrogen metabolism of sheep given maize stover ad libitum. *Animal Feed Science and Technology* **69**(1-3): 233 – 240.

Meik, J.M., R.M. Jeo, J.R. Mendelson III & K.E. Jenks. 2002. Effects of bush encroachment on an assemblage of diurnal lizard species in central Namibia. *Biological Conservation* **106**: 29 – 36.

Midgley, J.J. & Ward, D. 1996. Tests for inducibility of thorn defences against herbivory must consider plant growth strategies. *African Journal for Range and Forage Science* **13**: 75 – 77.

Milton, S.J. & M.T. Hoffman. 1994. The application of state-and-transition models to rangeland research and management in arid succulent and semi-arid grassy Karoo, South Africa. *African Journal of Rangeland and Forage Science* **11**(1): 18 – 26.

Moleele, N.M. & J.S. Perkins. 1998. Encroaching woody plant species and boreholes: is cattle density the main driving factor in the Olifants Drift communal grazing lands, south-eastern Botswana? *Journal of Arid Environments* **40**: 245 – 253.

Moleele, N.M., S. Ringrose, W. Matheson & C. Vanderpost. 2002. More woody plants? The status of bush encroachment in Botswana's grazing areas. *Journal of Environmental Management* **64**: 3 – 11.

O' Leary, C.H. & D.W. Nyberg. 2000. Treelines between fields reduce the density of grassland birds. *Natural Areas Journal* **20**: 243 – 249.

Oba, G., E. Post, P.O. Syvertsen & N.C. Stenseth. 2000. Bush cover and range condition assessments in relation to landscape and grazing in southern Ethiopia. *Landscape Ecology* **15**: 535 – 546.

Olff, H. and M.E. Ritchie. 1998. Effects of herbivores on grassland plant diversity. *Trends in Ecology & Evolution* **13**: 261 – 265.

Palgrave, K.C. 1977. Trees of Southern Africa. C. Struik Publishers. Johannesburg. p 244.

Porporato, A., P.D'Odorico, F. Laio, L. Ridolfi, & I. Rodriguez-Iturbe. 2002. Ecohydrology of water-controlled ecosystems. *Advances in Water Resources* **25**: 1335 – 1348.

Peres, C.A. 1998. Rethinking tropical ecosystem management. *Trends in Ecology & Environment* **13**: 252 – 253.

Radford, I.J., D.M. Nicholas & J.R. Brown. 2001. Impact of prescribed burning on *Acacia nilotica* seed banks and seedlings in the *Astrebla* grasslands of northern Australia. *Journal of Arid Environments* **49**: 795 – 807.

Rohner, C. & D. Ward. 1997. Chemical and mechanical defense against herbivory in two sympatric species of desert *Acacia Journal of Vegetation Science* **8**: 717 – 726.

Sankaran, M., J. Ratnam & N.P. Hanan. 2004. Tree-grass coexistence in savannas revisited insights from an examinations of assumptions and mechanisms invoked in existing models. *Ecology Letters* **7:** 480 – 490.

Savadogo, P., D. T. L. Sawadogo & M. Tigabu. 2008. Herbaceous species responses to long-term effects of prescribed fire, grazing and selective tree cutting in the savanna-

woodlands of West Africa. *Perspectives in Plant Ecology, Evolution and Systematics* **10**(3): 179 – 195.

Savadogo, P.D., M. Togabu, L. Savadogo & P.C. Oden. 2009. Examination of multiple disturbances effects on herbaceous vegetation communities in the Sudanian savanna woodland of West Africa. *Flora Morphology, Distribution Functional Ecology of Plants* **204**(6): 409 – 422.

Scholes, R.J. 1997. Savanna. *In* Cowling, R.M., D.M. Richardson and S.M. Pierce (eds). Vegetation of Southern Africa. Cambridge University Press. United Kingdom. pp 258 – 277.

Schultz, A.M., J.M. Launchbauch and H.H. Biswell. 1955. Relationship between grass density and brush seedling survival. *Ecology* **36**: 290 – 238.

Shacklton, C.M. 2000. Comparison of plant diversity in protected and communal lands in the Bushbuckridge lowveld savanna, South Africa. *Biological Conservation* **94**: 273 – 285.

Shrestha, M.K., A. Golan-Goldhirsh & D. Ward. 2002. Population genetic structure and the conservation of isolated populations of *Acacia raddiana* in the Negev Desert. *Biological Conservation* **108**: 119 – 127.

Skarpe, C. 1990. Shrub layer dynamics under different herbivore densities in an arid savanna, Botswana. *Journal of Applied Ecology* **27**: 873 – 885.

Smit, G.N. & N.F.G. Rethman. 2000. The influence of tree thinning on the soil water in a semi-arid savanna of southern Africa. *Journal of Arid Environments* **44**: 41 – 59.

Smit, G.N. 2004. An approach to tree thinning to structure southern African savannas for long-term restoration from bush encroachment. *Journal of Environmental Management* **71**(2): 179 – 191.

Strauss S.Y., J.A. Rudgers, J.A. Lau & Irwin RE. 2002. Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution* **17**: 278 – 284.

Stuart-Hill, G.C. & N.M. Tainton. 1988. Browse and herbage production in the Eastern Cape Thornveld in response to tree size and defoliation frequency. *Journal of Grassland Society of Southern Africa* **5**: 42 – 47.

Stuart-Hill, G.C. & N.M. Tainton. 1989. The competitive interaction between *Acacia karoo* and the herbaceous layer and how this is influenced by defoliation. *Journal of Applied Ecology* **26**: 285 – 298.

Teague, W.R. & G.N. Smit. 1992. Relations between woody and herbaceous components and the effects of bush-clearing in southern African savannas. *Journal of the Grassland Society of Southern Africa* **9**: 60 – 69.

Testerink, C., R.M. Van-der-Meulen, B.J. Oppedijk, A.H. De-Boer, S. Heimovaara-Dijkstra, J.W. Kijne and M. Wang. 1999. Differences in spatial expression between 14- 3-3 isoforms in germinating barley embryos. *Plant Physiology* **121**: 81 – 88.

Tews, J, A. Esther, S.J. Milton & F. Jeltsch. 2006. Linking a population model with an ecosystem model: Assessing the impact of land use and climate change on savanna shrub cover dynamics. *Ecological Modelling* **195**(3-4): 219 – 228.

Thiele, T., F. Jeltsch & N. Blaum. 2008. Importance of woody vegetation for foraging site selection in the Southern Peid Babbler (*Turdoides bicolor*) under two different land use regimes. *Journal of Arid Environments* **72**(4): 471 – 482.

Tredydte, A.C., I.M.A. Heitkonig, H.H.T. Prins & F. Ludwig. 2007. Trees improve grass quality for herbivores in African savannas. *Perspectives in Plant Ecology, Evolution and Systematics* **8**(4): 197 – 205.

Treydte, A.C., I.M.A. Heitkonig & F. Ludwig. 2009. Modelling ungulate dependence on higher quality forage under large trees in African savannahs. *Basic and Applied Ecology* **10**(2): 161 – 169.

Twyman, C. 2001. Natural resource use and livelihoods in Botswana's Wildlife Management Areas. *Applied Geography* **21**: 45 – 68.

Van Auken, O.W., E.M. Gese and K. Connors. 1985. Fertilization response of early and late successional species: *Acacia smallii* and *Celtis laevigata. Botanical Gazette* **146**: 564 – 569.

Van Auken, O.W. 2000. Shrub invasions of semiarid grasslands. *Annual Review of Ecology and Systematics* **31**: 197 – 216.

Van Auken, O.W. 2009. Causes and consequences of woody plant encroachment into western North American grasslands. *Journal of Environmental Management* **90**(10): 2931 – 2942.

van Wyk, B. & P. van Wyk. 1987. Field guide to Trees of Southern Africa. Struik Publishers. Cape Town. pp 484.

Vilela, A.E. & D.A. Ravetta. 2001. The effect of seed scarification and soil-media on germination, growth, storage, and survival of seedlings of five species of *Prosopis* L. (Mimosaceae). *Journal of Arid Environments* **48**: 171 – 184.

Walk, J.W. & R.E. Warner. 1999. Effects of habitat area on the occurrence of grassland birds in Illinois. *American Midland Naturalist* **141**: 339 – 344.

Walker, B.H. & W.T. Knoop. 1987. The response of the herbaceous layer in a dystrophic *Burkea Africana* savanna to increased levels of nitrogen, phosphate and potassium. *Journal of the Grassland Society of Southern Africa* **4**: 31 – 34.

Walter, H. 1939. Grassland, savanne, und busch der ariden teile Afrikans in ihrer ökologischen. *Bedingtheit Jaarboek Wissenschaftliche Botanie* **87**: 750 – 860.

Ward, D. 2005. Do we understand the causes of bush encroachment in African savannas? *African Journal of Range & Forage Science* **22**(2): 101 – 105.

Ward, D. and C. Rohner. 1997. Anthropogenic causes of high mortality and low recruitment in three *Acacia* tree taxa in the Negev desert, Israel. *Biodiversity and Conservation* **6**: 877 – 893.

Wendel, J.F. & N.F. Weeden. 1989. Visualization and Interpretation of Plant Isozymes. In: Soltis, D.E. & Soltis, P. (eds.). Isozymes in Plant Biology: Advances in plant science series. Vol. 4. Dioscorides Press, Oregon. pp. 5 – 45.

Wickens, G.E., A.G. Seif El Din, G. Sita & I. Nahal. 1995. Role of *Acacia* species in the rural economy of dry Africa and the Near East. FAO. Italy. pp 13 - 18.

Winter, M. & J. Faaborg. 1999. Patterns of Area Sensitivity in Grassland-Nesting Birds. *Conservation Biology* **13**: 1424 – 1436.

Witkowski, E. & R. Garner. 2000. Spatial distribution of soil seed banks of three African savanna woody species at two contrasting sites. *Plant Ecology* **149**: 91 – 106.

Chapter 3

POPULATION GENETICS OF BUSH ENCROACHING *Acacia mellifera*, AT PNIEL, NORTHERN CAPE PROVINCE, SOUTH AFRICA.

Abstract.

Based on spatial separation and different habitat qualities two populations of *Acacia mellifera* were observed in and around the town of Pniel, the vegetation of which is a semi-arid savanna, and is situated near Kimberley in the Northern Cape province of South Africa. One population appeared on rocky, andesitic laval ridges along the Vaal River. The other grows in a neighbouring sandveld area. The sites were located inside communal and commercial farms and a neighbouring game farm, which was on sandveld. For comparisons, an out-group population was sampled about 40km north of Pniel. Bush encroachment by *A. mellifera* was found to be more extensive on the rocky areas than in the sandveld. Seeds were sampled spatially on a logarithmic scale, in eight different sites (5 in the rocky habitat and 3 in the sandveld), for allozyme analysis. The allozymic data showed no significant difference between the two populations. Random genetic differences in the two populations, due to different environmental and pedological conditions were suspected to be responsible for the observed phenotypic differences. *A. mellifera* individuals within a geographic distance of 1 meter (rametes) from each other showed a very high level of genetic similarity with each other compared to those further away. This observation was evident across habitats. Nei's UPGMA dendrogram revealed the game farm subpopulations to be more primitive and genetically related. Despite differences in allozyme frequencies, between the sampled sites, genetic differentiation was found to be low (F_{ST} = 0.337). Nei's (1972) original measure of genetic distance ranged between 0.871 and 1.000 with a mean of 0.949 ± 0.053. Heavy bush encroachment seemed encouraged by sexual reproduction and the overall direction of

encroachment appeared to be eastward. Finally, in the light of the lack of genetic differences, we postulate that phenotypic differences may be the result of differences in ecohydrological and soil chemical properties, with different grazing histories also appearing to be involved.

Key words: Acacia mellifera, *bush encroachment, population differentiation, allozymes, vegetative and sexual reproduction.*

3.1 INTRODUCTION

Savannas are systems largely dominated by two vegetation strata viz. woody layer and the herbaceous layer, which is dominated by grasses (Hagos & Smit, 2005; Bond & Parr, 2010). The dominance of these two life forms can alternate such that a dynamic situation exists where neither of the two permanently dominates the other (Liedloff *et al.* 2001; Laclau *et al.* 2002; Sankaran *et al*. 2005). On the one hand, there exist a phase when the grassland dominates the system and such a savanna system is understood to benefit a range of biodiversity features and human dependency on savannas has been shown to peak during this phase (Smit, 2004). Human dependency includes activities such as subsistence and commercial farming (crop farming, livestock & game farming). Bush encroachment, on the other hand, refers to a transition when the density of woody plants increases at the expense of grasslands and the systems changes from an open system (grassland dominant) to a closed system (dominated by the woody layer) (Ward, 2005; Van Auken, 2009). Although the exact factors that cause the system to shift from one phase to another are still not clearly understood, many hypotheses have been brought forward and some of these highlight rainfall amounts, disturbance that reduces the density of one life form and thus give the other an advantage to increase in density (e.g. human activities that result in clearing of woody plants), the combination of grazing and rainfall and/or competition for soil nutrients as possible explanations (Ward, 2005; Tews *et al.* 2006; Zida *et al.* 2007; Bond & Parr, 2010).

In spite of all the research that has been done, no general explanation has been drawn which can explain the phenomenon across all savanna systems of the world as more findings have only been site-specific.

Within encroaching species, which, in Africa includes *Acacia* spp. and other woody legumes, phenotypic differences are evident when the species is encroaching in different environmental conditions (Hempson *et al.* 2007; Mboumba & Ward, 2008). This has often been explained as gene-by-environment or local adaptation whereby environmental conditions resulted in certain genes being activated and thus leading to certain phenotypic traits being expressed (Mboumba & Ward, 2008). The expression of certain phenotypes, under different environmental conditions results in different populations of the same species being evident and this may further lead to population differentiation if the two created populations genetically drift apart over time as was the case in *Colophospermum mopane* (Villeon *et al.* 2003).

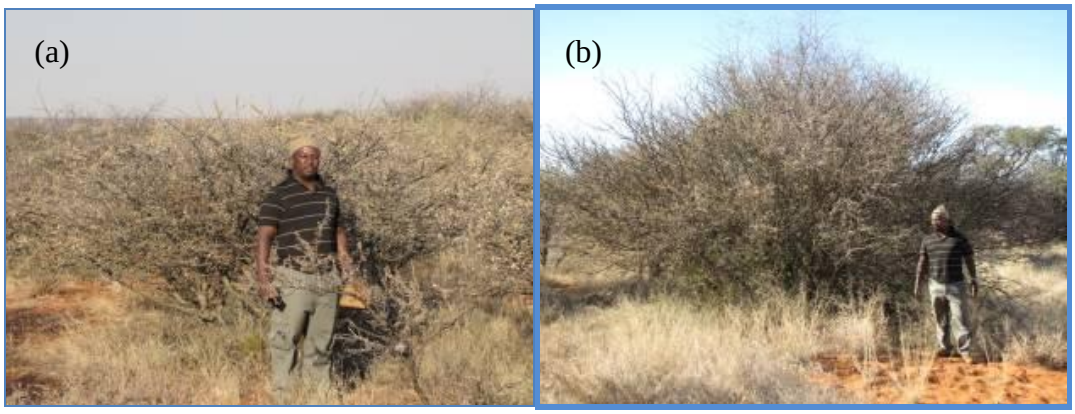

Figure 3.1: Different stature of *Acacia mellifera* trees growing in a rocky habitat and sandveld conditions . *A. mellifera* trees in rocky areas showed stunted growth and formed dense, impenetrable thickets across the habitat (a) whereas in the sandveld, they formed clusters of relatively taller trees (b).

According to Sultan (2000) a single genotype within a population can be expressed in different phenotypic forms in different environments, a plant characteristic known as

phenotypic plasticity (Sharma & Esler, 2008; West-Eberhard, 2008). Reasonably, plants mostly being sessile have to be genotypically plastic in order to cope with changing environments (Schlaepfer *et al.* 2002; Ellis *et al.* 2006; West-Eberhard, 2008). Comparative, quantitative genetics and molecular approaches have been acknowledged in leading new insights into the adaptive nature of plasticity, its underlying mechanisms and its role in the ecological distribution and evolutionary diversification of plants (Storfer, 1996; Sultan, 2000; Pigliucci, 2005; Ellis *et al.* 2006).

According to Reed and Frankham (2001) it is the ability of a population to undergo adaptive evolution, which allows populations to differentiate and adapt to new landscape variations. With molecular genetics as an appropriate tool in determining genetic differentiation and quantitative genetics for analyzing G x E interaction, it is beneficial to combine both molecular genetics and quantitative genetics in order to test if truly phenotypic plasticity is adaptive (Pigliucci, 2005; Sultan & Stearns, 2005; Ellis, 2006).

To illustrate the concept of G x E, it might be worth looking at an effect of local-scale as reflected in the findings of Hempson *et al.* (2007), on a study on an arid savanna, in Kruger National Park of South Africa, two divergent growth forms of *Colophospermum mopane* were noted. One comprised of tall, single stemmed trees and the other of short, multi-stemmed shrubs and both forms were found growing in the riparian and savanna ecological zones. Using inter-simple sequence repeat (ISSR) amplification profiles to test if the two growth forms were genetically distinct, results indicated that they were not genetically distinct but rather the observed differences were environmentally determined (Hempson *et al.* 2007). The phenomenon of gene/environment interaction has been documented before, the environment affects the ways in which genes are expressed so that genes switched on in one condition may be downregulated in other environments (Ellis *et al.* 2006; Chauhan *et al.* 2007). Intriguingly, sometimes a control gene that positively affects

another gene in one environment may have the opposite effect in another environment (Pigliucci, 2005; Ellis *et al.* 2006; Chauhan *et al.* 2007). Therefore, genetic plasticity is important as otherwise populations might be forced to extinction.

Another study on the relationship between genetic correlation and genetic distance on *Chlamydomonas*, showed on the one hand that the quantity of G x E increased as both the environmental variance across environments and the genetic distance increased (Kassen & Bell, 2000). On the other hand, the genetic correlation declined as the environmental variance between pairs of environments and the genetic distance between pairs of genotypes increased (Kassen & Bell, 2000). This suggested that the divergent selection would be more likely to maintain genetic variation when environments are strongly contrasted and the genotypes widely divergent.

Genetic markers have made it much easier to identify quantitative trait loci (QTL), which are said to be the chromosomal regions or individual sequence variants responsible for trait variation (Barton & Keightley, 2002). This genetic breakthrough has a major potential role in ascertaining population differentiation and probably in correlating local adaptation with fitness. Once population differentiation has been confirmed and the factors that cause it identified, conservation measures can then be explored.

In the study area, near Kimberley in South Africa, two populations of *Acacia mellifera* subsp. *detinens* were observed. The main population occurring at very high densities comprised of *A. mellifera* individuals of low stature (see Figure 3.1a), above) and was found on rocky andesitic laval ridges alongside the Vaal River. A second population occurred sporadically in the sandveld away from the river, consisting of individuals of higher stature (Figure 3.1b). Bush encroachment was far more common in the rocky habitat. The soil types in the two habitats differed considerably, particularly in pH (the sandveld had a pH_{KCl} of 4 whilst the rocky habitats ranged from 6.50 to 7.00). Therefore the capacity of the andesitic

soils to retain nutrients was assumed to be far greater than that of the sandveld. In spite of encroachment being so undesirable, it might be ecologically detrimental to exterminate one or both of the populations if population differentiation has taken place.

3.2 MATERIALS & METHODS

3.2.1 The Study Area

The study area in Pniel (28°35'126" **S**; 24°32'248" **E**) near Kimberley, in the Northern Cape province of South Africa is typical of savanna showing encroachment of *Acacia mellifera* as described in Chapter 2 (also see Figure 3.2). Consequently sampling for this study was done here. The two populations were found on andesitic laval ridges near the Vaal River, and within sandy flats further south. The sandveld had a pH_{KCl} of 4 whilst the rocky habitats ranged from 6.50 to 7. Significantly more fine material (clay plus silt) was evident in soils of the rocky area (23.95%) than the sandy sites (16.12%; $p<0.01$, t-test). Collection of seeds was carried out at eight sites in the Pniel area. Details on sites are listed in Table 3.1. The sites differed in habitat type (sandveld or rocky) as well as farming management practiced (Table 3.1 and Figure 3.2). The game farm site was located on both habitat types.

Table 3.1: Habitats and type of farming management on sites where collection of *A. mellifera* seeds was conducted.

Site	**Management**	**Habitat Type**	**No. of Trees Sampled**
Game farm (rocky pop.)	Game farming	Rocky	17
Game farm (sandy pop.)	Game farming	Sandveld	16
Rocky pop.2	Commercial	Rocky	17
Sandy pop.2	Commercial	Sandveld	14
Community area (rocky pop.)	Communal	Rocky	13
Rocky pop.4	Commercial	Rocky	17
Rocky pop.5	Commercial	Rocky	17
Sandy pop.3	Commercial	Sandveld	13
Windsorton*	Commercial	Rocky	8

*an out-group

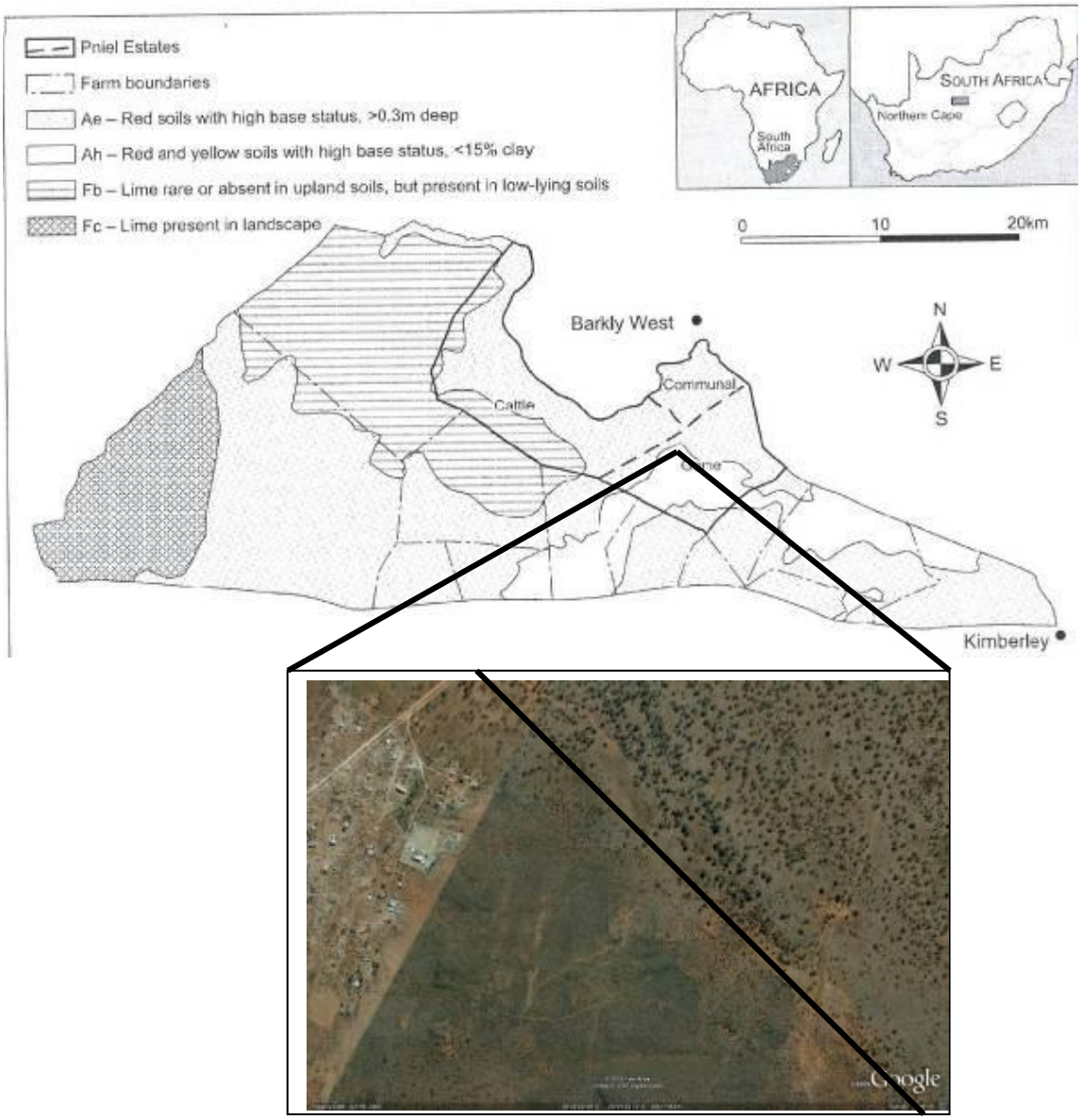

Figure 3.2: Map of the Pniel Estates, study site, and an insert of a satellite image of the game farm depicting two scenarios of bush encroachment by *Acacia mellifera* subsp. *detinens*, on either side of the diagonal line. On the top-right is bush encroachment in a sandveld area (encroachment appears in clusters within a grassland) and on the bottom-left, is encroachment on a rocky area (encroachment forms continuous thickets of *A. mellifera*). (Background image from Britz & Ward (2007); satellite image from GoogleEarth).

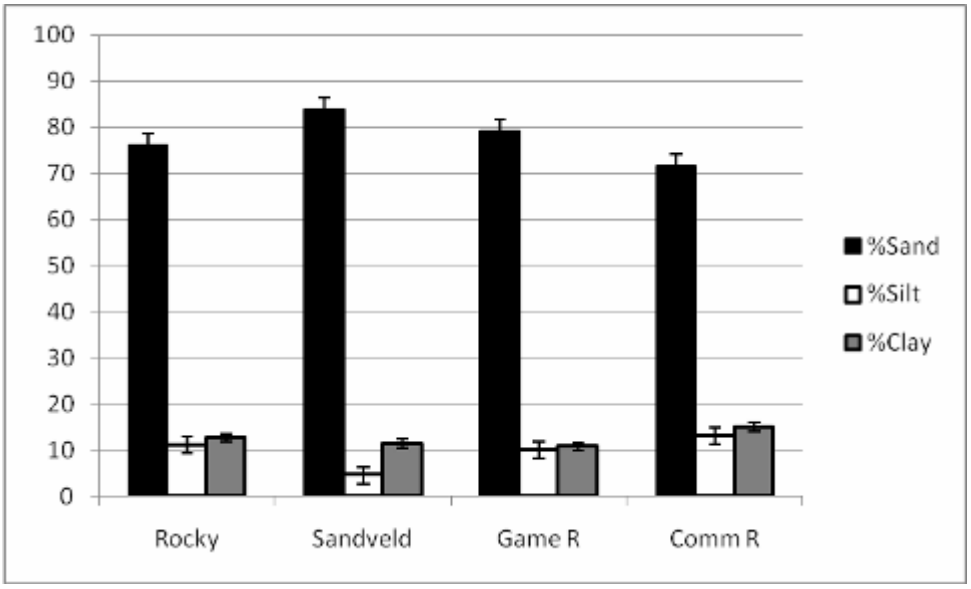

Figure 3.3: Percentage of soil particle size distribution between rocky and sandveld habitats where bush encroachment of *Acacia mellifera* subsp. *detinens* was observed. (NB: Game R denotes a game farm on rocky habitat. Comm R denotes a communal land on rocky habitat).

3.2.2 Seed Collection for Starch Gel Electrophoresis (SGE)

Collection was done at the eight sites described in Table 3.1, during November 2001. In each of the habitat types (sandveld or rocky) a target tree, a large reproductive tree was chosen and seeds collected from it. From each target tree, neighbouring trees in the four cardinal directions, with the target tree acting as the centre point, were sampled. Neighbouring trees were sampled at intervals on a logarithmic scale at 1, 10, 100 and 1000 m away from the target tree (Figure 3.4). This was designed with the aim of covering as much of the encroached area in each site, as possible and to further assist in determining the scale of genetic population differentiation. This design also allowed for the determination of whether ramets (any independent individual plant originating from a sexually-induced seed or derived by vegetative reproduction) found in close proximity to each other were clones of the target tree. A total of 139 trees were sampled for starch gel electrophoresis, from the 8 different sites (Table 3.1).

Seeds were collected from all of these trees with the exception of 2 from which vegetative tissue, leaves, was obtained. For comparison, seeds from *A. mellifera* were sampled at Windsorton (28^{o}20'. 630" **S**: 024^{o}46'. 861"**E**), which in spite of being rocky had no encroachment. Windsorton lies about 40 km north of Pniel. Here eight trees were sampled along a road transect. The distance between each of these trees was measured and these trees formed an out-group for comparison. These seeds were used in conducting starch gel electrophoresis, which separates out different individuals on the basis allozyme characteristics (i.e. frequency). All seeds collected were frozen at –80^{o} C until extraction and starch gel electrophoresis analysis.

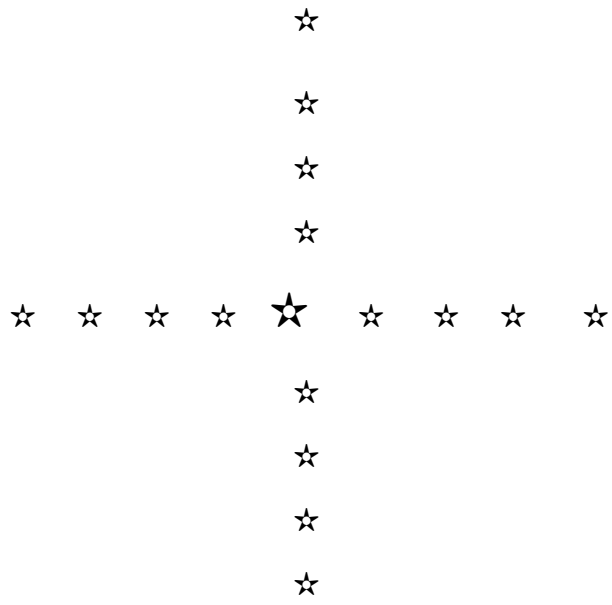

Figure 3.4: Sampling design for seed collection from *A. mellifera* in encroached areas of Pniel. Trees were sampled in the 4 cardinal directions from a target tree (centre). The neighbouring trees were at intervals 1, 10, 100 & 1000 m away from the target tree.

3.2.2.1 Starch Gel Electrophoretic Analysis of Seeds.

Seeds of *A. mellifera* from an encroached savanna, in Pniel, were analyzed using starch gel electrophoresis which entails electrophoretic separation of allozymes (variant forms of proteins or gene products). This separation can be achieved through starch gel electrophoresis

(SGE), which was used in this study, polyacrylamide gel electrophoresis (PAGE), agarose gels, and cellulose-acetate membranes. Starch gel electrophoresis was chosen over all the others because of the following advantages, as mentioned by Wendel & Weeden, (1989):

1. It allows for the analysis of large numbers of individuals for several to many different enzymes,
2. It has a much higher resolving power compared to agarose and cellulose-acetate gels
3. Up to six enzyme systems can be analyzed from a single gel, while only one can be analyzed with PAGE gels.

However, there are disadvantages to the technique when compared to PAGE gels. With PAGE, for instance, changing the concentration of acrylamide can vary the stringency of molecular sieving (Wendel & Weeden, 1989).

Extracts were made from the collected seeds from the sampled trees (n = 137). However, in the two cases where seeds were unavailable, vegetative tissue (leaves) was used. For analysis, seeds were explants of choice because literature shows that much enzyme activity is found here after imbibition but this has also been reported in metabolically, young active leaves (Wendel & Weeden, 1989).The endosperms of seeds were ground into fine powder and transferred with an extraction buffer, Tris [Tris(hydroxymethyl) aminomethane] (See Appendix I), into Eppendorf tubes in order to effect adequate cell breakage. The application of an appropriate extraction buffer plays an important role in minimizing the complexing of enzymes after cellular disruption. In particular it minimizes interference from substances like phenolics (Wendel & Weeden, 1989). However, no extraction buffer can be guaranteed to be optimally effective in protecting all the enzymes present (Wendel & Weeden, 1989). After homogenization, the extracts were kept at -80^0C overnight. Extracts were centrifuged before use, the following morning, in order to exclude any debris.

3.2.2.2 Allozyme Analysis

A 12% concentrate of starch gels (See Appendix I) were prepared a day before use. Each gel was prepared as per buffer requirements (See Appendix I). After preparation, the gels were kept in the refrigerator until the following day. The following day, an incision on the gel cutting from end to end was made about 3 cm from the bottom. After centrifugation of the seed extracts, Whitman No. 1 paper wicks (2 x 10 mm) were dipped into each extract and then inserted into the gel, 1-2 cm apart. At each extremity, a paper wick dipped in Bromophenol Blue was inserted and this was used as a marker to gauge the distance travelled by the samples. The gels were run for 8 hours, after which they were sliced into thin slices ready to be soaked in enzyme treatments (See Appendix I). After treatment, the treated slices were kept in darkness overnight, at room temperature and the bands appearing in the different lanes were measured using a ruler. In each gel run, the target tree of a site and a group of neighbouring trees were electrophoresed together. Therefore, electrophoresis experiments were done through Starch gels, in three different buffers and stained with six different enzymes (Table 3.2).

Table 3.2: Buffers and enzymes used to conduct Starch Gel Electrophoresis of the sampled *A. mellifera* seeds. (For more details on the buffers, see Appendix I).

Buffers	**Enzymes**
Ridgeway (discontinuous)*	*Glucose-6-phosphate dehydrogenase* (G6PGH)
	Glucose-6-phosohate isomerase (GPI)
TC-Buffer (pH 6.9)	*Peptidase {L-leucylgylcylglycine}* (PEP-LGG)
	Fructose biphosphate aldolase (FBA)
TBE-Buffer (pH 9.0)	*Phosphoglucomutase* (PGM)
	Diaphorase {cytochrome b5 reductase} (DIA)

*Gel & electrode buffer have different pH's (Gel = pH 8.7 & Electrode = pH 8.0)

After Starch Gel Electrophoresis, a number of different allozymes were detected from *A. mellifera* seeds using the different enzymes in different buffers.

Statistical analysis.

The interpretation of allozyme patterns in *Acacia mellifera* is extremely difficult because of its ploidy level (tetraploid with 2n = 4x = 52 chromosomes), as indicated by Oballa (1993) and, Oballa and Olng'otie (1992). Data were treated as phenotypes, scoring the presence or absence of bands representing alleles (e.g. Brain, 1985, 1989; Shrestha *et al*. 2002). The following parameters indicating genetic diversity were described:

the proportion of polymorphic loci (*% P*), obtained by counting the number of loci with ≥ 2 bands (alleles) for total number of loci within each population;

mean number of alleles per polymorphic locus (*AP*), and

mean number of alleles per locus (*A*).

We used POPGENE 1.31 to calculate the Shannon index of gene diversity (*H*) and Nei's genetic distance (*D*) (1972). The multivariate relationships among individuals and between populations was analyzed using a Principal Coordinate Analysis (PCoA) using the Multi-Variate Statistical Package (MSVP- Kovach, 1999). The Mantel Nonparametric Test package in GenStat-sixth Edition, version 6.1.0.200, by Lawes Agricultural Trust, (Copyright ©2002), was used to test population differentiation in terms of genetic distance within and between the subpopulations in the two different habitats. The Mantel Test uses two methods, which determine the significance using the standard normal variate (g) and using random permutations of the first matrix to determine the possible variation within the data (Mantel, 1967). A t-test was performed on the level of genetic similarity between the individuals and the geographic distance to see if the two observed populations were significantly different.

3.3 RESULTS

3.3.1 Allozyme Analysis

In order to study the population genetics of this particular acacia species, of which two populations were observed in the study area (Pniel, Kimberley), allozyme analysis was chosen as a method of choice and this required a simple approach in terms of collecting material, and it could be accomplished by using seeds. After electrophoresis with six different enzymes, in three different buffers, a number of different allozymes were detected.

Table 3.3: Number of various allozymes detected in the seeds of *Acacia mellifera*, sampled from eight localities and electrophoresed through the Ridgeway buffer (a discontinuous buffer: Electrode buffer, pH 8.0 & Gel buffer pH 8.7) and stained with six different enzymes.

Locality	G6PDH	GPI	pep-LGG	FBA	PGM	DIA
Game farm (sandy pop.)	6	2	13	2	2	3
Game farm (rocky pop)	3	6	4	2	2	9
Rocky pop.2	1	2	3	1	2	2
Sandy pop.2	1	1	2	1	1	1
Community area (rocky pop)	1	1	1	1	1	2
Rocky pop.4	3	3	6	1	1	1
Rocky pop.5	1	1	1	1	1	11
Sandy pop.3	1	1	2	1	1	1
Windsorton*	-	1	2	1	1	1

* = out-group

However, performing electrophoresis on the seed extracts on different buffer systems, TC buffer (pH 6.9) and TBE buffer (pH 9.0), and analyzing the banding patterns with the same set of enzymes, yielded different sets of allozymes, (see the following Tables).

Table 3.4: Number of various allozymes detected in the seeds of *Acacia mellifera* from the different localities as electrophoresed through the TC buffer (pH 6.9) and stained with the six different enzymes.

Locality	G6PDH	GPI	pep-LGG	FBA	PGM	DIA
Game farm (sandy pop.)	3	3	2	2	2	3
Game farm (rocky pop.)	6	18	2	2	3	10
Rocky pop.2	1	2	2	1	2	2
Sandy pop.2	1	2	1	1	1	1
Community area (rocky pop.)	4	1	1	1	1	1
Rocky pop.4	2	2	4	1	1	1
Rocky pop.5	1	2	2	1	1	1
Sandy pop.3	1	2	2	1	1	1
Windsorton*	1	1	2	1	1	1

* = out-group

Table 3.5: Number of various allozymes detected in the seeds of *Acacia mellifera* from the different localities as electrophoresed through the TBE buffer (pH 9.0) and stained with the six different enzymes.

Locality	G6PDH	GPI	pep-LGG	FBA	PGM	DIA
Game farm (sandy pop.)	2	3	1	1	2	2
Game farm (rocky pop.)	4	4	2	2	2	4
Rocky pop.2	2	1	2	1	1	1
Sandy pop.2	1	1	1	1	1	1
Community area (rocky pop.)	1	1	2	1	1	1
Rocky pop.4	1	1	2	1	1	1
Rocky pop.5	1	1	1	1	1	1
Sandy pop.3	1	1	1	1	1	1
Windsorton*	1	1	1	1	1	1

*=out-group

Finally, all allozymes detected were added up according to their respective habitat source. Windsorton, although being a rocky habitat, was treated separately simply because it was an out-group (no encroachment reported at Windsorton). The so-obtained allozyme summations were then tabulated against each of the six enzymes used (Table: 3.6).

Table 3.6: Average number of different allozymes from *Acacia mellifera*, as detected through six different enzymes. Sampling was done into different habitats, rocky and sandveld (Windsorton, an out group was also included).

	Sandveld		Rocky		Windsorton	
	Sum	Avg	Sum	Avg	Sum	Avg
G6PDH	17	5.667	32	10.667	2	1.000
GPI	16	5.333	46	15.333	3	1.000
PEP-LGG	25	8.333	35	11.667	5	1.667
FBA	11	3.667	18	6.000	3	1.000
PGM	12	4.000	21	7.000	3	1.000
DIA	14	4.667	48	16.000	3	1.000

3.3.2 Analysis of Genetic Similarity

Genetic similarity, as represented by allozymes of the different "mother plants" was analyzed using a Multivariate Statistical Package (MVSP version 3.13c, 1985 – 2002 Kovach Computing Services). This analysis allowed for comparison of the different variates (allozyme) at more than one variable (gene loci) with data interpreted on axes in multidimensional space. The comparison further enables for an effective reduction in dimensionality from say 2 to 1, by representing the sample data according to its index of size:

$$y_1 = x_1 \cos\partial + x_2 \sin\partial$$

$$y_2 = -x_1 \sin\partial + x_2 \cos\partial$$

where x_1 and x_2 are different variates or allozymes of the same individual or gene loci (Krzanowski, 1988). In this way, the analysis provides the best expression of the relationship between the sample points in a two dimensional graphical representation (Green, 1978; Krzanowski, 1988). The data thus obtained is displayed in Figure 3.5, which is the level of genetic variability between the "mother plants" from the nine different sites. From this data, no significant difference was noted (PCOA, $p > 0.05$), as the “mother plants” were not distinctly separated in the two axes.

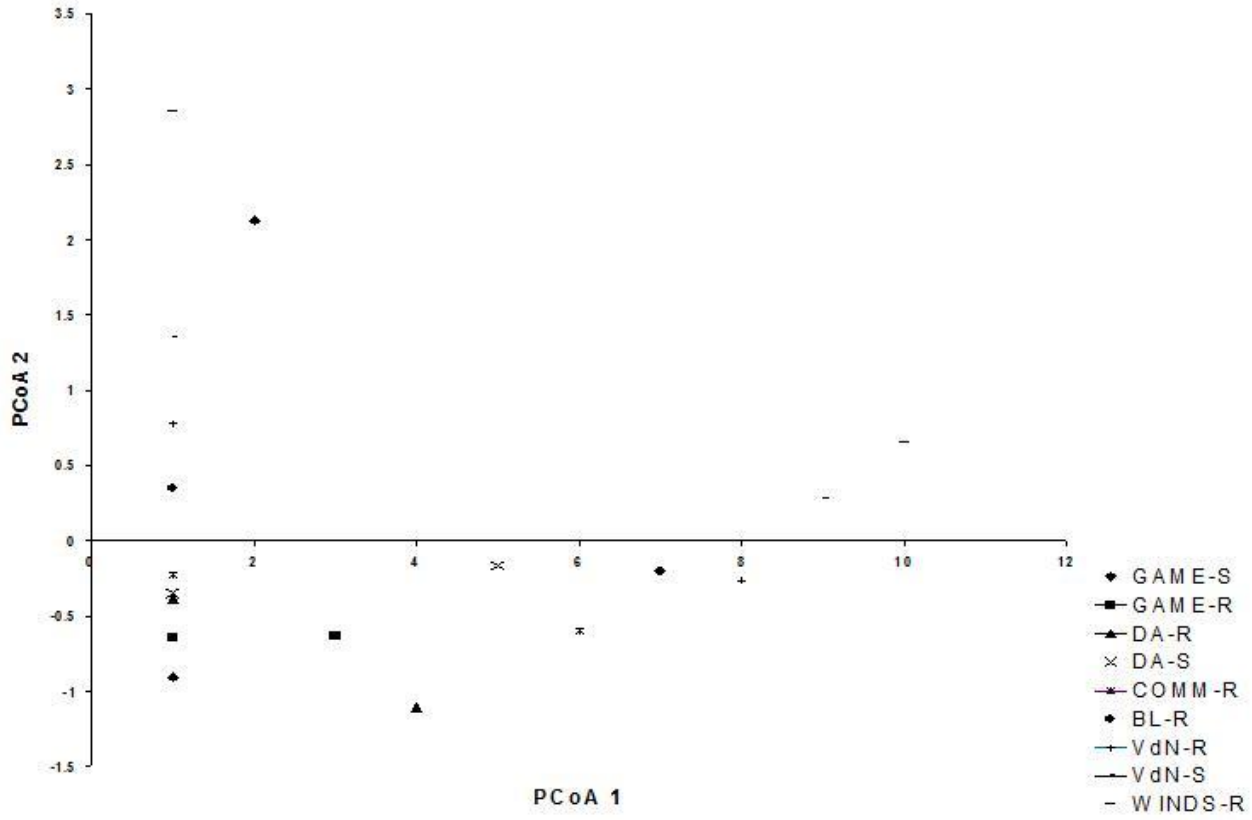

Figure 3.5: Comparison of allozyme variability between the "mother plants", as sampled from different sites in the rocky and sandveld habitats. [Key: GAME-S = game farm (sandveld pop.); GAME-R = game farm (rocky pop.); DA-R = rocky pop.2; DA-S = sandy pop.2; COMM-R = community area (rocky pop.); BL-R = rocky pop.4; VdN-R = rocky pop.5; VdN-S = sandy pop.3].

Furthermore, genetic variability was measured by comparing all the allozymes from each particular site, within a habitat. All the allozymes detected in one site were pooled to represent that particular site, as defined by a mother plant of that particular site. Still using MVSP, genetic variability between the eight sites was compared and once again, no significant difference on either of the axis was noted (PcoA, $p > 0.05$), (Figure 3.6).

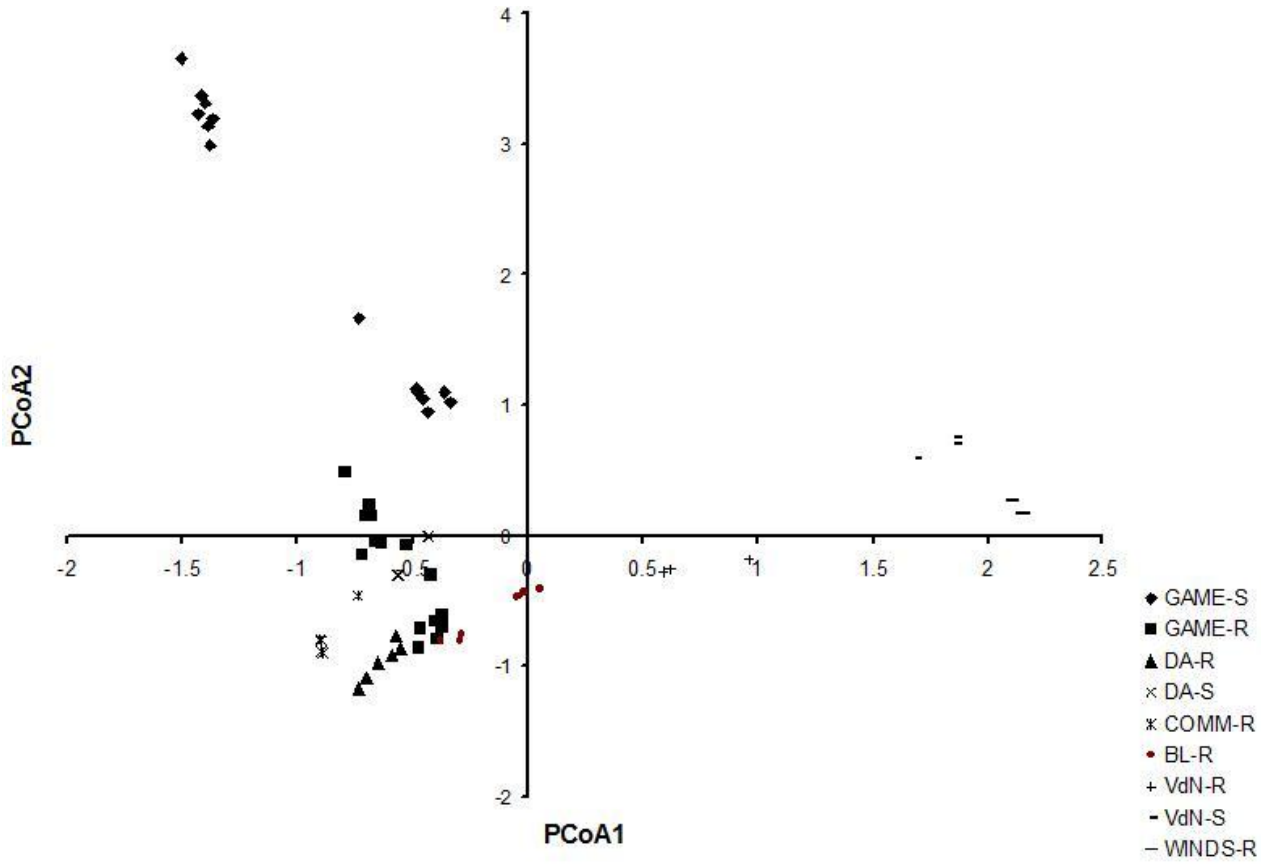

Figure 3.6: Comparison of allozyme variability between the eight different sites, three of which were found in the sandveld. (*note*: Windsorton, an out-group was included). {Key: GAME-S = game farm (sandveld pop.); GAME-R = game farm (rocky pop.); DA-R = rocky pop.2; DA-S = sandy pop.2; COMM-R = community area (rocky pop.); BL-R = rocky pop.4; VdN-R = rocky pop.5; VdN-S = sandy pop.3}.

Further genetic comparison was done by pooling all the allozymes detected in each habitat, and the two habitats (sandveld and rocky area) were then compared using Principal Component Analysis. Once again, no genetic significant difference was observed (PcoA, $p > 0.05$), Figure 3.7.

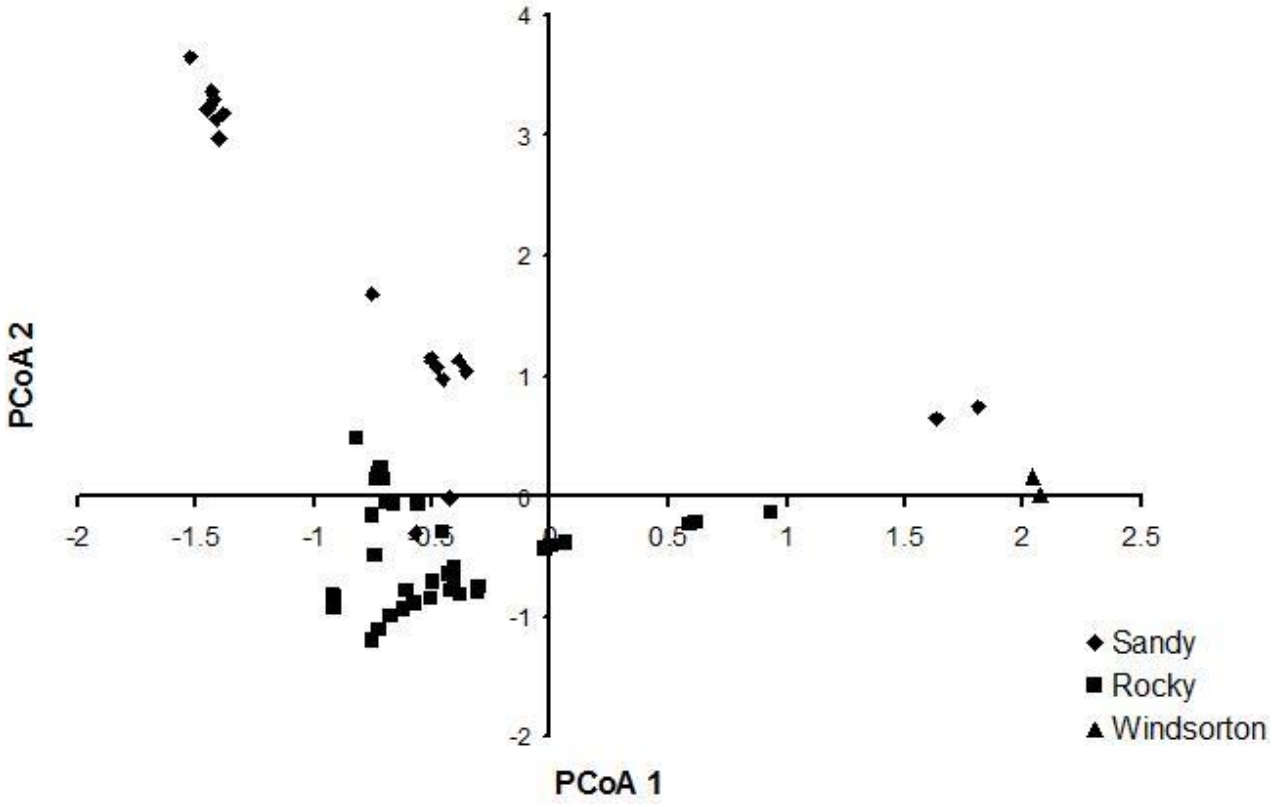

Figure 3.7: Allozyme comparison between the two habitat types. Once again, no significant difference between the two habitats was noted as the habitat types could not be separated out . (*note*: Windsorton, an out-group was included).

Since on each of the sites a different type of farm management was practiced, the nine sites studied were grouped according to farm management. When genetic similarity between the two observed populations was measured to determine if farm management had an effect on genetic variability, no genetic significant difference was noted (PcoA, $p > 0.05$), Figure 3.8.

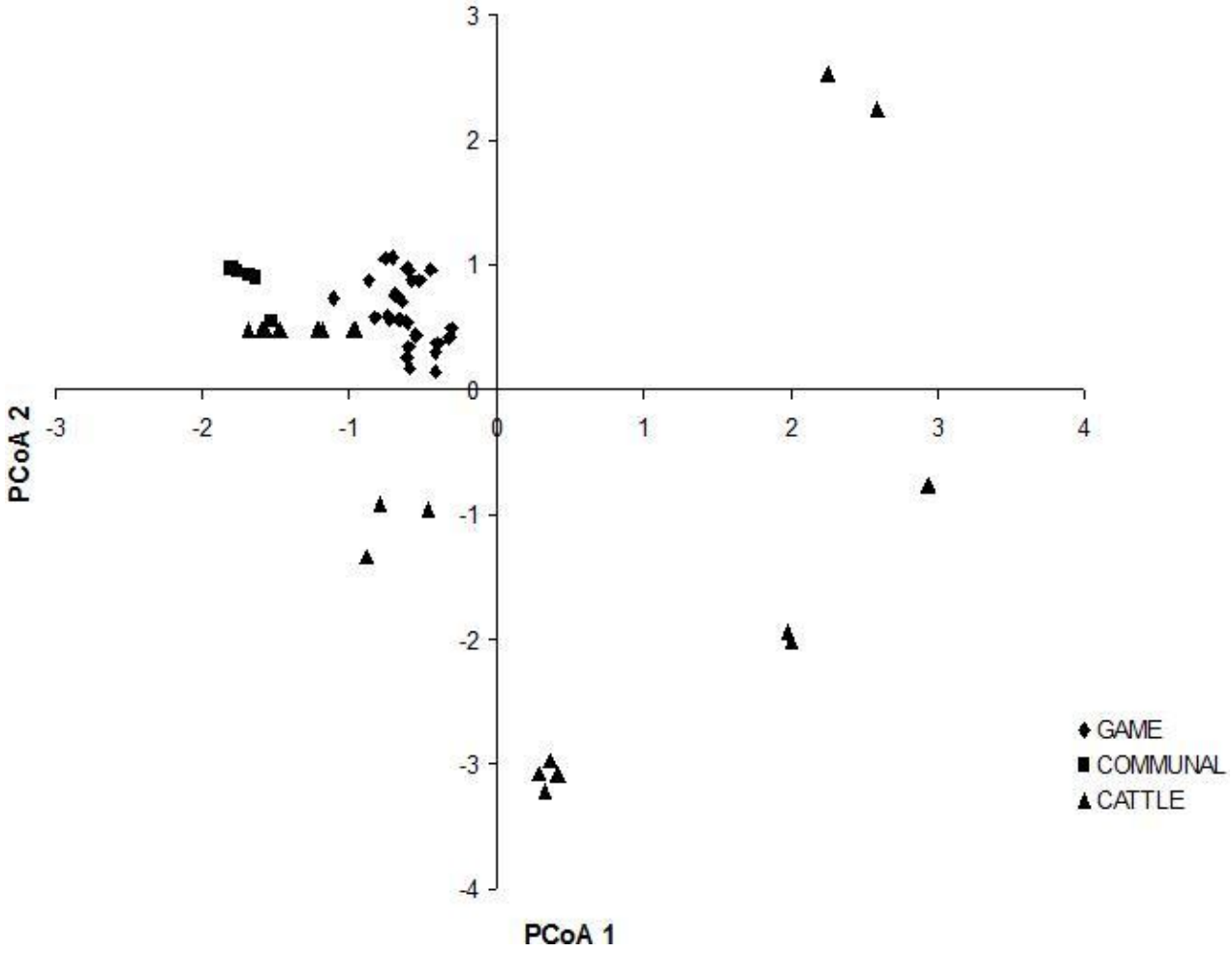

Figure 3.8: Comparison of allozyme variability in *Acacia mellifera* populations growing under different types of farm management, viz; game farming, communal farming and cattle farming. Farm management induced no significant difference in genetic variability of the subpopulations (PCoA, $p > 0.05$).

Table 3.7: Summary table for significant difference on genetic variability between the two observed populations of *Acacia mellifera,* in a sandveld habitat and the other in a rocky habitat.

Genetic Variability	***p*-level**
Between "mother " plants	>0.05
Between the eight sampled sites	>0.05
Between the two habitats	>0.05
Between farm management types	>0.05

3.3.3 Allozyme Frequency

Establishing which habitat had the highest genetic variability (lowest genetic similarity) could be indicative of which mode of reproduction would most likely be taking place in that particular habitat. High genetic variability (low genetic similarity) would be indicative of sexual reproduction and low genetic variability (high genetic similarity), could be associated with vegetative reproduction. The rocky habitat where bush encroachment was documented to be worse than in the sandveld was found to have high genetic variability (Figure 3.9). This high level of allozyme variability was followed by that of the sandveld, which had mild encroachment, and the least variability was noted in Windsorton (an out-group), which in spite being rocky had no encroachment and not much sampling was done there.

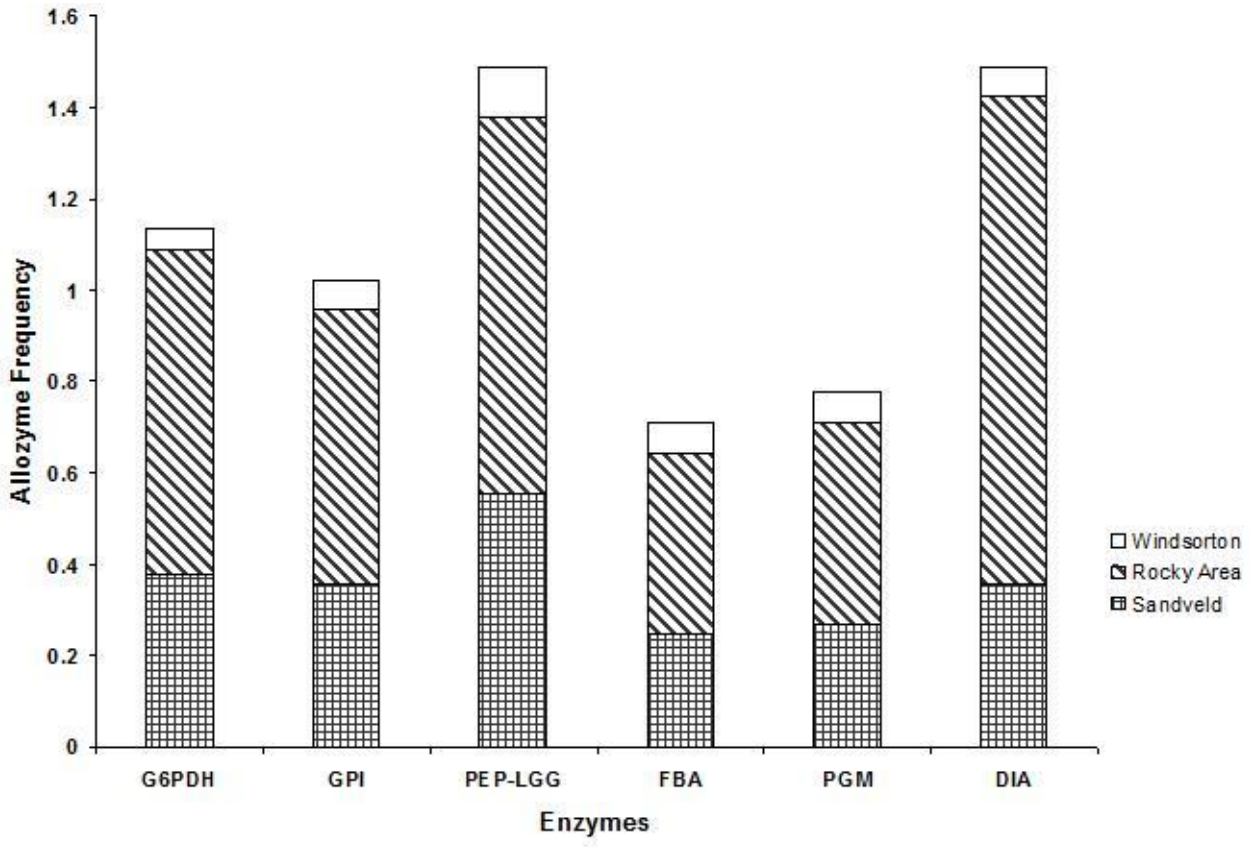

Figure 3.9: Depiction of allozyme frequency as a reflection of the number of allozymes detected in each habitat. NB: At Windsorton, an out-group, in spite of being rocky no encroachment was noted.

Detection of many allozymes in a habitat could be associated with a particular mode of reproduction. For instance, many different allozymes (high genetic variability or low genetic similarity) detected in an area might indicate sexual reproduction as a probable, dominant mode of reproduction in that particular area. Thus a relative low number of allozymes (low genetic variability or high genetic similarity) could be associated with vegetative reproduction. In order to determine the probable mode of reproduction that *Acacia mellifera* could be propagating by in a particular habitat, the average number of allozymes (i.e. the average of the sums of allozymes detected through each of the three buffers), detected in a habitat was calculated and these were compared between habitats (Figure 3.10).

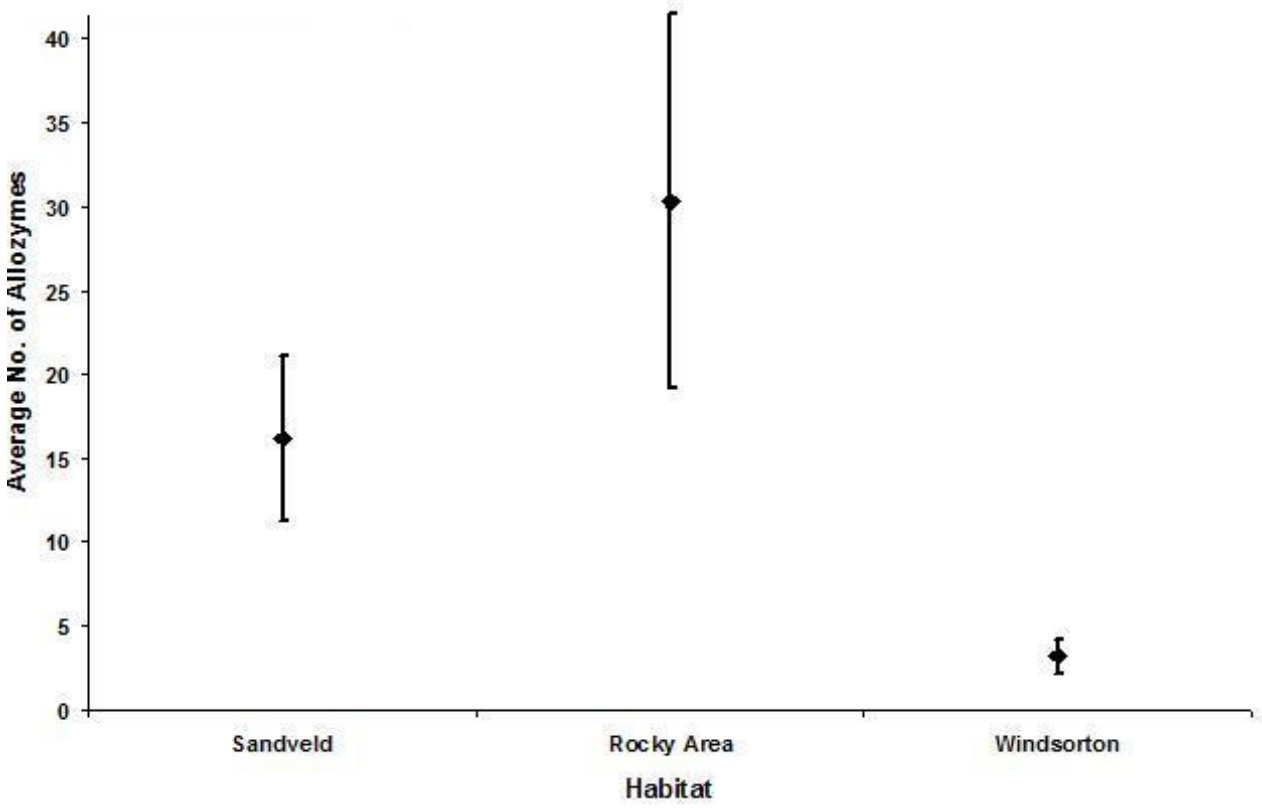

Figure 3.10: Average number of allozymes detected in each habitat representative of genetic variability which further solicits the probable mode of reproduction in that particular habitat. (Error bars represent the level of variation)

3.3.4 Directional Spread of Encroachment by **Acacia mellifera**

When a plant species is encroaching an area, its encroachment might be spreading in a particular direction, which may be important in determining the cause of bush encroachment such as that by *Acacia mellifera* at Pniel. Knowing the direction of spread might also be very fruitful in predicting which other areas are more likely to be encroached. Such knowledge could also be useful in planning management strategies for areas not yet encroached but most likely to be.

In order to determine the most probable direction of spread, firstly a scatter-plot of all the "mother" plants was plotted and from this, the central "mother" plant was identified. All the other "mother plants" were then assigned the direction in which they laid relative to the central "mother" plant, to the nearest 45^{o} angle of the absolute direction (North, South, West & East). Then the total number of allozymes detected in the all the as represented by their "mother plants" was added together and finally added to the number of allozymes found in the respective direction of the central "mother plant" individual (Chen *et al.* 2009). From this data, a scatter plot of the number of allozymes per direction was plotted (Figure 3.11).

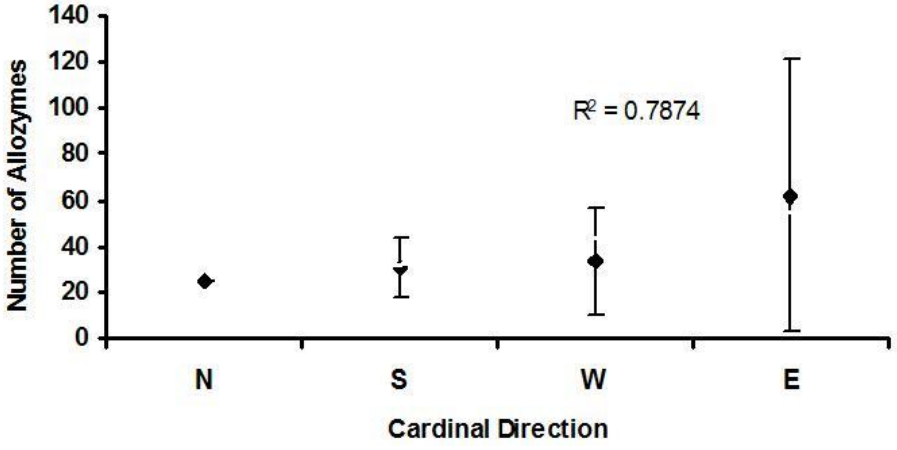

Figure 3.11: Probable direction of spread of encroachment, by *Acacia mellifera*, as represented by the average number of allozymes detected in a particular direction. (NB: Low genetic similarity associated with sexual reproduction and thus a higher probability of heavy encroachment, and high genetic similarity, with vegetative reproduction).

Measuring the level of genetic similarity between the *A. mellifera* individuals, between the different subpopulations showed that they were significantly different from each other ($F_{(5, 407)} = 30920.110$, $p < 0.01$, see Table 3.8).

Table 3.8: Comparing the level of genetic similarity between the individuals of *Acacia mellifera,* between the rocky subpopulations showed that they were significantly different from each other, on the basis of genetic similarity.

d.f Effect	MS Effect	d.f Error	MS Error	F	p-level
5	1962.753	407	0.063	30920.110	<0.001

To test if the mean level of genetic similarity obtained in each of the eight sampled subpopulations (Figure 3.12), an ANOVA test at a 95% Confidence Limit, was carried out and it was discovered that the eight sampled subpopulations were not significantly different from each other ($F_{(7, 1013)} = 1.451$, $p = 1812$).

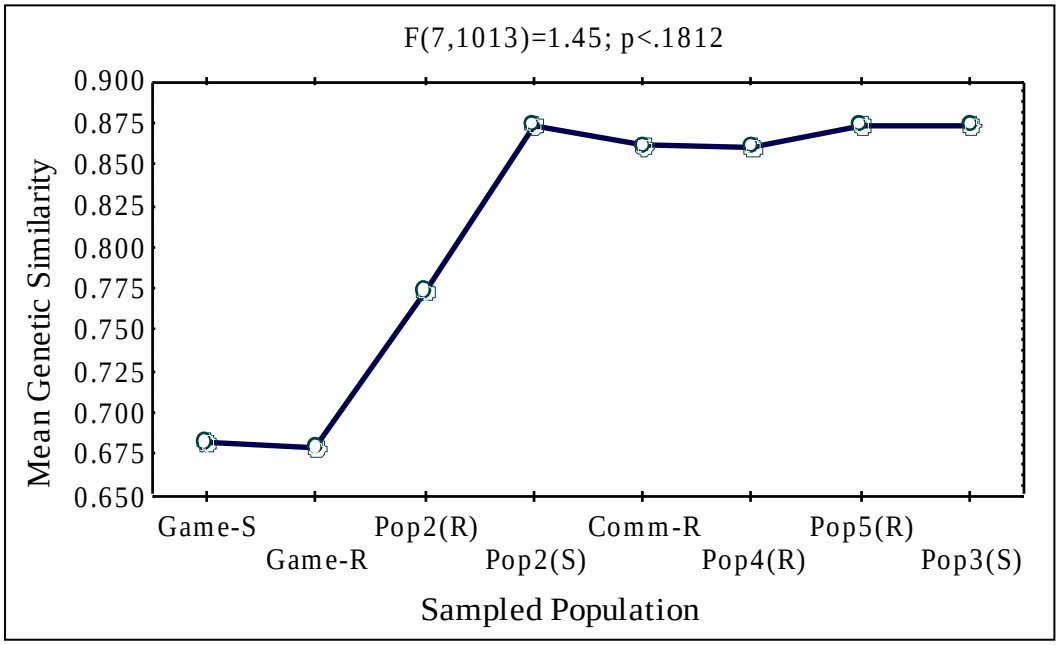

Figure 3.12: The test of mean genetic similarity between the eight sampled sites showed that the game farm subpopulations were significantly different from the other subpopulations (ANOVA, $F_{(7, 1013)} = 1.540$, $p < 0.1812$) as also shown in Nei's UPGMA dendrogram. Key: Game-S = game farm (sandy pop); Game-R = game farm (rocky pop); Pop2(R) = rocky pop2; Pop2(S) = sandy pop2; Comm-R = community area (rocky pop); Pop4(R) = rocky pop4; Pop5(R) = rocky pop5; Pop3(S) = sandy pop3.

A test was then done to verify if the mean genetic similarity differences plotted in figure 3.12, were in fact significantly different and it was shown they were not (Table 3.9).

Table 3.9: A test of mean genetic similarity observed in each of the sampled subpopulations revealed that they were not significantly different from each other.

df Effect	MS Effect	df Error	MS Error	F	p-level
7	6.697	1013	4.615	1.451	>0.05

Table 3.10: Summary table of genetic similarity between *Acacia mellifera* individuals as measured per different characteristics.

Attribute measure	R^2-value	*p*-level
By cardinal direction	0.7874	>0.05
By "mother" plant in sandveld	0.1831	
By "mother" plant in rocky areas	0.6068	>0.05
By sandy habitat	0.0011	
By rocky habitat	0.0809	>0.05

3.3.5 F-statistics on Genetic Similarity

An *F* statistics on the genetic similarity that was observed between the *Acacia mellifera* individuals was measured using PopGene in order to understand the level of heterozygosity between and within the observed populations of *A. mellifera.* The *F* statistics (F_{IS}, F_{IT} *and* F_{ST}) for the two populations of *A. mellifera* are shown in Table 3.15, for 6 variable loci.

Table 3.11: Summary of *F*-statistics at all loci of nine sampled areas (including the out-group) encroached by *Acacia mellifera.*

Locus	F_{IS}	F_{IT}	F_{ST}	N*m*
GPI-1	0.579	0.709	0.301	0.557
G6P-2	0.260	0.460	0.269	0.678
PEP-3	-0.140	0.234	0.328	0.512
FBA-4	1.000	1.000	0.393	0.385
PGM-5	1.000	1.000	0.410	0.360
DIA-6	0.456	0.688	0.313	0.550
Mean	0.456	0.640	0.337	0.492

The values of F_{IS}, which is a measure of deviation from the expected number of heterozygous genotypes per locus, ranged from 0.149 to 1.000 and averaged 0.628. F_{ST}, is a measure of genetic differentiation relative to the subpopulation and these statistics served to reflect how the two observed populations measured against expected heterozygous genotypes and also to highlight measured differentiation against the sampled subpopulations. Values of F_{IT}, inbreeding relative to the two observed populations, varied from 0.234 to 1.000 with an average of 0.682. There were no negative F_{IS} or F_{IT} values, which would be indicative of an excess of heterozygotes (Evans, 1987).

Wright's (1978) fixation index (F_{IS}) as a measure of heterozygote deficiency or excess, between the populations, averaged 0.628. This average, 0.628, indicated that 68.2% of the variance in allelic frequencies between the populations is explained by the geographic site of the population. The loci that contributed most to increasing the F_{IS} values were FBA-1 and PGM-1, which represented 34% and 23%, respectively, of the possible among ramete variability in allelic frequencies. Shannon's information index (Lewontin, 1972) on the genetic variation for all loci had a mean of 0.412 ± 0.058, (Table 3.12).

Table 3.12: Shannon's information index (Lewontin, 1972) on the genetic variation for all loci.

Locus	*Shannon's information index*
GPI-1	0.420
G6P-1	0.407
PEP-1	0.507
FBA-1	0.354
PGM-1	0.433
DIA-1	0.349
	0.412
Mean	
St. Dev	0.058

N*m*, a measure of gene flow estimated from F_{ST} was calculated to be 0.492 after a Chi-square test (Table 3.13) for each of the six loci used, was conducted. The analysis implied that the amount of gene flow, from one population to the other, received by each population is less than half, per generation.

Table 3.13: Chi-square test, of the six loci used in the allozyme analysis, for deviation from Hardy-Weinberg proportions in the nine samples areas (including the out-group) encroached by *Acacia mellifera.*

Locus	***p*-level**
GPI-1	<0.001
G6P-2	<0.001
PEP-3	0.003
FBA-4	<0.001
PGM-5	<0.001
DIA-6	<0.001

3.3.6 Nei's Genetic Distance

Nei's (1972) original measures of genetic distance ranged between 0.871 and 1.000 with a mean of 0.949 ± 0.053. The clustering of the various samples of *Acacia mellifera* using the unweighted pair group method with arithmetic averages (UPGMA) is shown in Figure 3.13.

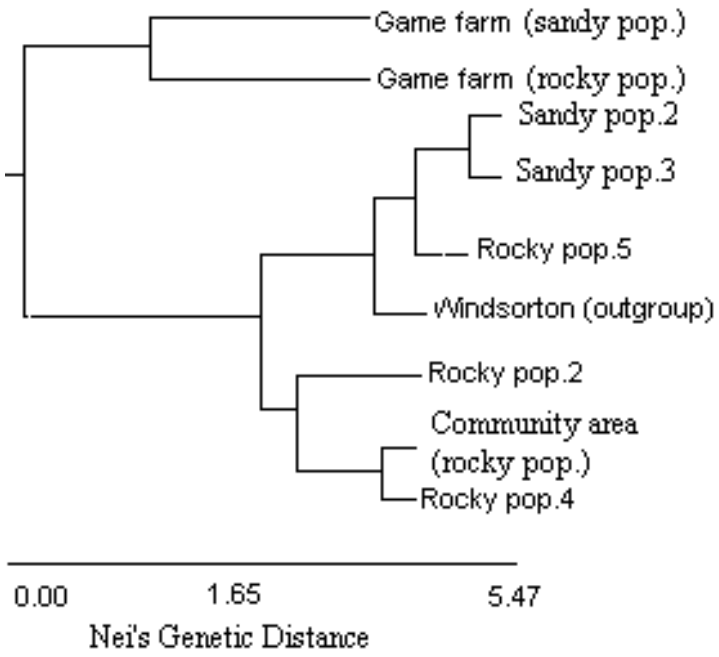

Figure 3.13: Unweighted pair group method with arithmetic averages (UPGMA) dendrogram using Nei's (1972) genetic distance between the nine sampled areas (including the out-group which was not encroached), which were encroached by *Acacia mellifera.*

3.3.7 *Population Differentiation*

(i) *Mantel Nonparametric Test*

Population differentiation in terms of genetic distance within and between the subpopulations in the two different habitats was tested using the Mantel Nonparametric Test package in GenStat-sixth Edition, version 6.1.0.200, by Lawes Agricultural Trust, Copyright ©2002. The Mantel Test uses two methods, which determine the significance using the standard normal variate (g) and using random permutations of the first matrix to determine the possible variation within the data (Mantel, 1967). In this analysis, 100 permutations were set after data conversion into matrices, using GenStat. The data was converted into two 8 x 8 matrices, one for geographic distance and the other for genetic distance. The geographic and genetic matrices had means of 7.605 and 0.8492, respectively. A null hypothesis (H_o), that there is no association between the elements in the dissimilarity matrix1 (geographic

distance) and the dissimilarity matrix2 (genetic distance), was tested. The results obtained are shown in Table 3.14.

Table 3.14: Mantel Test for population differentiation and level of correlation between genetic and geographic matrices.

Nature of test	**Tested variable**	**level**
Mantel Test based on sums of squares	Association between geographic and genetic matrices	212.000
	Percent permutations with equal or greater association	94.00
Mantel Test based on product-moment correlations	Association between geographic and genetic matrices	-0.3434
	Percent permutations with equal or greater association	94.00

The H_o that there is no association between the elements in the disimilarity matrix1 and disimilarity matrix2 was thus accepted at the 95% Confidence Limit as $p > 0.05$ (Mantel Test). Furthermore, a negative correlation (Mantel Test, $r = -0.3434$) between the two matrices was, noted implying that for an increase in geographic distance, there was a decrease in genetic similarity between *Acacia mellifera* individuals. Alternatively, this negative correlation confirms the observed trend that individuals, which were within short geographical distance, had higher genetic similarity than those further away, i.e. a decrease in geographic distance corresponded to an increase in the level of genetic similarity (Figs. 3.9, 3.10 and 3.12).

(ii) *Two Sampled T-test*

A t-test was performed on the level of genetic similarity between the individuals and the geographic distance to see if the two observed populations were significantly different. The t-test results are shown in Table 3.15.

Table 3.15: Two-Sample T-test performed at a 95% Confidence Limit on level of genetic similarity (first two rows) and on geographic distance (last two rows).

Habitat	**Size**	**Mean**	**Variance**	*F-level*	**d.f**	**p-level**	**t-level**	**d.f**	**p-level**
Rocky	40	0.816	0.026	1.030	39	0.960	0.03	62	0.978
Sandveld	24	0.815	0.025		23				
Rocky	40	8.445	34.930	1.270	39	0.500	-0.59	62	0.557
Sandveld	24	9.390	44.440		23				

With regard to the level of genetic similarity, the p-value for the null hypothesis of equal variances was found to be p = 0.960. The test statistic t came out to be 0.03 and the 95% Confidence interval was from –0.081 to 0.083 with the p-value of 0.978. Looking at the populations in terms of geographic distances, the p-value for the null hypothesis of equal variances was 0.500. In this case a negative value for the test statistic t was –0.59 and the 95% Confidence interval ranged from –4.146 to 2.255, with a p-value of 0.557. As can be noticed, in both cases the populations were not significantly different.

3.4 DISCUSSION

After observing two populations of *Acacia mellifera* subsp. *detinens* in the Pniel, Kimberley, Northern Cape province of South Africa, allozyme data was interpreted using a multidimensional visualization technique, reification, to test for genetic similarity between the two populations. The objective was to separate the two populations on different axes of the principal coordinate system and this would establish genetic differences between the two populations. Based on allozyme variability analyses, as revealed by the PCoA projections, comparisons between "mother" plants, the eight sampled subpopulations, farm management types and the two habitats in which the observed two populations of *A. mellifera* were observed, our results could not genetically separate the two populations.

A Shannon-Weaver index of allozyme vatiability, between the observed two populations, was also measured to test if there was a significance difference in terms of

allozyme variability. This index is a measure of diversity within species of populations implemented to measure diversity in categorical data and is simply the information entropy of the distribution. The index would have increased had there been additionally more unique allozymes in one of the two populations. However, it was less than 0.500 ($H = 0.412 \pm 0.058$) indicating low allozyme richness and hence no significance difference between the observed populations. A two sample t-test, assuming homogenous variance within each population, was also carried out to evaluate sample means of allozyme variation between the two observed populations and this also yielded no significant difference.

In determining whether there was any significant genetic difference between the two observed populations of *A. mellifera*, no significant difference could be established. Even at relatively large landscape scales, it appeared that the level of genetic similarity remained highly conserved. Although there is not much genetic research conducted specifically on *A. mellifera* elsewhere, for comparison purposes, Stanton *et al*. (2002), also made the same observation in their study on *Artemisia.* They found that even though the taxa they studied appeared quite distinct both morphologically and ecologically, extremely low levels of genetic divergence were observed. Similar findings were also made in the Kruger National Park where two divergent growth forms of *Colophospermum mopane* (Kirk ex Benth) Léonhard were noted (Hempson *et al.* 2007). One was short, multi-stemmed and the other was of tall trees. Once again, no genetic differentiation was confirmed after believing the two constituted two different populations, rather G x E interactions came through.

Therefore, the two populations of *A. mellifera*, observed in Pniel (study area) could not be confirmed as genetically distinct populations. Rather, environmental conditions should have had significant impacts in activating certain genes in one habitat and not in the other. This lack of genetic differentiation between the observed two divergent growth forms of *A. mellifera* might be as a result of different local environmental conditions, G x E interactions

and access to deeper water associated with soil depth (Fraser *et al.* 1987; O'Connor, 1992; Mapaure, 1994; Hempson *et al.* 2007). Phenotypic plasticity was therefore more probable rather than genetic differentiation or local adaptation and to confirm this, a completely-crossed design experiment was recommendable. In such an experiment one could sample seeds from one habitat and plant them in the other habitat and see if their response will differ from their source counterparts.

The structural differences observed between the two habitats suggested a high probability for different modes of reproduction taking place within each population. On the one hand the rocky population appeared in dense, stunted thickets that covered vast areas of the rocky habitat. On the other hand, the sandveld population appeared in sporadic clusters dotted as islands within the grasslands of the sandveld. As a result, is was speculated that for individuals to appear in isolated clusters, vegetative reproduction could have led to such a spatial distribution as opposed to sexual reproduction which easily would result in an evenly distributed spread of seedlings, in space (typical of the rocky habitat scenario).

Although no significant different results could be shown in terms of the level of genetic similarity between the sandveld population and rocky area population, a high level of genetic similarity would have been indicative of vegetative reproduction whereas low genetic similarity would suggest sexual reproduction (Infante *et al*. 2003; Martin *et al*. 2003). This too would confirm the spatially clustered and uniform thicket appearance of *A. mellifera* associated with observations made in the sandveld and rocky areas of the study area, respectively. However, in general our study showed no significant difference in terms of genetic similarity and therefore it could not be conclusively established that the two populations were in fact reproducing via different reproductive modes, sexual vs vegetative mode. On the one hand sexual reproduction allows for genetic assortment and recombination of different genetic material from different individuals to take place and thus a low level of

genetic similarity or high allozyme variability is accomplished (Guenete & Bonhomme, 2003). On the other hand, with vegetative reproduction, allozyme variability will be low or genetic similarity will be high because the F1 generation of a particular "mother" plant are a direct result of its genetic make-up (Infante *et al*. 2003; Martin *et al.* 2003).

High allozyme variability was noted in the rocky areas of Pniel, which can be attained through sexual reproduction. The second highest allozyme variability was noted in the sandveld where mild encroachment was noted and the least allozyme variation was noted in Windsorton. *A. mellifera* in the sandveld appeared to be propagating through vegetative reproduction as it occurred in clusters and the level of genetic diversity was low (Cruz & Moreno, 2001). The low level of allozyme variation or high genetic similarity noted in this study validates this notion. Therefore, in areas with high clay and silt content (and the presence of rocks), *A. mellifera* might engage in sexual reproduction. Subsequently, encroachment manifests itself and high allozyme variation can thus be detected (Guenete & Bonhomme, 2003). On the contrary, in sandy areas where there are low silt and clay contents, and deep soils, individuals of *A. mellifera* might reproduce vegetatively. The absence of rocks in the sandveld, which trap water in the rock-soil interfaces after rains and thus make water available for longer periods, thus amounts to a reduction in water retention (Mackay, 2001; Britz & Ward, 2007). As a result water and soil nutrients should leach straight down to the water table much quicker than in the rocky areas (high silt content), thus restricting the level of encroachment that can be attained in the sandveld (Saunders *et al.* 1997). In rocky areas, the availability of water for longer periods, the absence of grasses (disruption of the two-layer strata) and the availability of nutrients through nitrogen fixation (by the leguminous *A. mellifera*) and manure from herbivores, might all make conditions conducive for heavy encroachment to occur at a larger scale, as was observed in the study area.

When an area is transformed by bush encroachment, knowing the direction of spread is crucial in informing land-use management and facilitating decision-making in areas that are not yet encroached. Such knowledge could be used proactively in advising which farms and/or protected areas need to take adaptive or preventative steps. The direction of spread could be influenced by directional spread of seed dispersal, especially where sexual reproduction is prevalent. In turn, seed dispersal is facilitated by vectors such as wind (Schippers & Jongejans, 2005). Although the dominant seed dispersal vector for *A. mellifera* in the study area, is not known, depending on the resultant direction taken by the seed dispersal vector (probably wind), seed germination and bush-encroachment should also be in that direction. For the study, there seemed to be an overall trend of encroachment spreading towards the east. Therefore, strong southerly and westerly winds might be a contributing factor to the observed direction of spread, such that about 79% of the observed encroachment was explained by direction of spread. Therefore, assuming east as the direction of spread, farmers and land-use managers east of *A. mellifera* thickets should prioritize their management activities. However, the precise reasons underlying this directional spread, still need to be fully investigated.

A UPGMA dendrogram was plotted to age the subpopulations and the UPGMA revealed that the two subpopulations both subject to game farming (one subpopulation in the rocky habitat and the other in the sandveld) are more closely related to each other than with any of the other six subpopulations. This meant they probably branched off the main population around the same time. Although closely related and shown to have originated from the same main population, we conclude that the differential nature of the two populations in terms of their ability to encroach may be down to different environmental conditions. This once again underscored the effect local environmental conditions can have

on plant populations. The observed divergent growth forms could have arisen as a factor of phenotypic plasticity.

Thus my findings were similar to those of Hempson *et al.* (2007) wherein two divergent growth forms of *Colophospermum mopane* were observed growing in different environments and population differentiation was hypothesized. However, findings revealed no population differentiation, no local adaptation but rather phenotypic plasticity. In the case of my findings, *A. mellifera* appeared did not only occur in two different environments, it also appeared to be regenerating differently as one population showed signs of reproducing sexually whilst the other showed signs of reproducing vegetatively. Therefore, it was not only different growth forms being observe that were divergent but even reproductive strategies implemented in each environment appeared to be divergent. In addition, given the observed structural and reproductive differences, the UPGMA dendrogram based on Nei's genetic distance revealed two particular population to be of the same age and having branched off the main population around the same time. These findings have implications for control of this bush encroaching species in savannah regions.

Certainly, curbing bush encroachment by *A. mellifera* might not be an easy task. In Botswana a study on controlling bush encroachment by *Acacia nigrescens* was conducted using fire and it was found that fire can control bush up to a height of 2 meters but regrowth is very much likely if no follow-up measures are taken (Sweet & Tacheba, 1985). However, it might be worthwhile to first know what kind of a population one is dealing with, i.e. in terms of propagation. If a population is not propagating through sexual reproduction (where low encroachment and high genetic similarity between different individuals might be occurring), the application of bio-controls that target seeds or reproduction might prove a waste of money and highly ineffective. Rather, the application of mechanical means like up-rooting seedlings or cut/treat stumps might be fruitful. In other words, any means that

destroys the roots of the plant or prevents coppice formations should be beneficial. However, where a population has engaged in sexual reproduction, up-rooting individuals might be a waste of time but rather the application of bio-control treatments like Bruchid beetles or any other means that targets and destroys the seeds could be highly recommended and productive.

3.5 CONCLUSION

As *Acacia mellifera* is said to be able to engage in both vegetative and sexual reproduction modes (Adams, 1967), this bimodal reproduction of *A. mellifera* was also observed in this study, though further studies may be necessary. Although it still remains unclear as to the conditions under which will *A. mellifera* propagate through either mode, there seem to be a possibility that clay and silt (and probably the presence/absence of rocks), soil pH, soil depth (water availability) and access to soil nutrients all play a pivotal role in determining the mode of reproduction. Despite the observed differences in allozyme frequencies among the eight sampled subpopulations of this study, the level of genetic differentiation was rather low (F_{ST} = 0.337). Therefore, it can be deduced that the two observed populations of *A. mellifera*, in Pniel (study area), are not significantly different and have not differentiated as the principal component analysis performed in this study also failed to separate them out as different populations. The performed Mantel test and the Two-sampled T-test also showed non-significant results confirming the two observed populations have not differentiated. Nonetheless, G x E interactions might have led to two observed growth forms and probably triggered different modes of reproduction.

Although there was found to be heavy encroachment in the rocky areas, except at Windsorton, some encroachment was also noted in the sandveld. The overall direction of spread was found to be toward the east. Despite the seed dispersal vector not being ascertained, it became more likely that wind might be influential in determining the direction

of spread, as opposed to animals, which should have no definitive direction. Knowing the direction of spread might be helpful in predicting which other areas are more likely to be encroached in the near future and who should worry about their land being invaded by *A. mellifera.*

3.6 FUTURE RESEARCH

Based on my results and in furthering the research on *A. mellifera*, it might be worthwhile to look at the following areas, in the future:

- Under what conditions and why would *A. mellifera* populations alternate modes of regeneration? Such information could be valuable in managing other populations elsewhere and also in recommending control treatments where populations are encroaching. Research on other acacia species has shown that species regenerating through sexual reproduction may be harder to control than those regenerating through root suckers (Munkert, 2009).
- Based on the assumption that the rocky habitat population propagated sexually whilst that in the sandveld habitat regenerated vegetatively, are seeds produced in one habitat already genetically programmed for one specific mode of reproduction? This will be important for population management.

3.7 ACKNOWLEDGEMENTS

I would like to thank Dr. Shayne Jacobs who became my supervisor after Professor D. Ward moved to UKZN. I also would like to thank the staff and students, of the years 2001 – 2002, of Conservation Ecology department of the University of Stellenbosch for their support and making my academic study at the University of Stellenbosch such a stimulating and awarding time. My ex-supervisor Professor David Ward for his commitment, encouragement and all

that he taught me. Dr. Colleen Downs of the University of Natal (Pietermaritzburg campus) for her immeasurable assistance with my write-up. To the National Research Foundation (NRF), Cape Tercentenary, Erickssen & Ethel Trust Foundation and the University of Stellenbosch for financial support.

3.8 LITERATURE CITED

Adams, M.E. 1967. A study of the ecology of *Acacia mellifera, A. seyal* and *Balanites aegyptiaca* in relation to land-clearing. *Journal of Applied Ecology* **4**: 221 – 273.

Barton, N.H. & P.D. Keightley. 2002. Understanding quantitative genetic variation. *Nature Reviews* **3**: 11 – 21.

Bond, W.J. & C.L. Parr. 2010. Beyond the forest edge: Ecology, diversity and conservation of the grassy biomes. *Biological Conservations* **143**(10): 2395 – 2404.

Brain, P., 1985. Leaf peroxidase types in *Acacia karroo*. Geographical distribution and influence of the environment. *South African Journal of Botany* **52:** 47 – 52.

Brain, P., 1989. Genetic races in a ring species of *Acacia karroo*. *South African Journal of Science* **85:** 181 – 185.

Britz, M-L & D. Ward. 2007. Dynamics of woody vegetation in a semi-arid savanna, with a focus on bush encroachment. *African Journal of Range & Forage Science* **24**(3): 131 – 140.

Chauhan, T., K.K. Lal, V. Mohindra, R.K. Singh, P. Punia, A. Gopalakrishan, P.C. Sharma & W.S. Lakra. 2007. Evaluating genetic differentiation in wild populations of the Indian major carp, *Cirrhinus mrigala* (Hamilton-Buchanan, 1882): Evidence from allozyme and mocrosatellite markers. *Aquaculture* **269**(1-4): 135 – 149.

Chen, Y., W. Yang, W. Li, Z. Li & H. Huang. 2009. High allozyme diversity and unidirectional linear migration patterns within a population of tetraploid *Isoetes sinensis*, a rare and endangered pteridophyte. *Aquatic Botany* **90**(1): 52 – 58.

Cruz, A., J.M. Moreno. 2001. No allocation trade-offs between flowering and sprouting in the lignotuberous, Mediterranean shrub *Erica australis. Acta Oecologia* **22:** 121 – 127.

Ellis, B.J., J.J. Jackson & W.T. Boyce. 2006. The stress response systems: Universal and adaptive individual differences. *Developmental Review* **26**(2): 175 – 212.

Fraser, S. W., van Rooyen T. H. & Verster E. (1987) Soil-plant relationships in the central Kruger National Park. *Koedoe* **30,** 19–34.

Guenet, J. & F. Bonhomme. 2003. Wild mice: an ever-increasing contribution to a popular mammalian model. *Trends in Genetics* **19**: 24 – 31.

Hagos, M.G. & G.N. Smit. 2005. Soil enrichment by *Acacia mellifera* subsp. *detinens* on nutrient poor sandy soils in a semi-arid southern African savanna. *Journal of Arid Environments* **61**(1): 47 – 59.

Hempson, G.P., E.C. February & A.G. Verboom. 2007. Determinants of savanna vegetation structure: Insights from *Colophospermum mopane. Austral Ecology* **32**, 429 – 435.

Infante, D., G. Gonzalez, L. Peraza-Echevarria & M. Keb-Llanes. 2003. Asexual genetic variability in *Agave fourcroydes. Plant Science* **164**: 223 – 230.

Kassen, R.S. & G. Bell. 2000. The ecology and genetics of fitness in Chlamydomonas.X. The relationship between genetic correlation and genetic distance. *Evolution* **54**: 425 – 432.

Laclau, J., W. Sama-Poumba, J. Nzila, J. Bouillet & J. Ranger. 2002. Biomass and nutrient dynamics in a littoral savanna subjected to annual fires in Congo. *Acta Oecologica* **23**: 41 – 50.

Liedloff, A.C., M.B. Coughenour, J.A. Ludwig & R. Dyer. 2001. Modelling the trade -off between fire and grazing in a tropical savanna landscape, northern Australia. *Environment International* **27**: 173 – 180.

Mackay, D.S. 2001. Evaluation of hydrologic equilibrium in a mountainous watershed: incorporating forest canopy spatial adjustment to soil biogeochemical processes. *Advances in Water Research* **24**: 1211 – 1227.

Mapaure I. (1994) The distribution of *Colophospermum mopane* (Leguminosae-Caesalpinioideae) in Africa. *Kirkia* **15,** 1–5.

Martin, A.P.M.S., L. Adamec, J. Suda, T.H.M. Mes & H. Storchova. 2003. Genetic variation within the endangered species *Aldrovanda vesiculosa* (Droseraceae) as revealed by RAPD analysis. *Aquatic Botany* **75**: 159 – 172.

Mboumba, G.B. & D. Ward. 2008. Phenotypic plasticity and local adaptation in extreme populations of *Acacia karroo. African Journal of Range and Forage Science* **25:** 121 – 130.

Munkert, H.C. 2009. Sexual and vegetative regeneration of three leguminous tree species in South African savannas. *South African Journal of Botany* **75:** 606 – 610.

O'Connor T. G. (1992) Woody vegetation – environment relations in a semi-arid savanna in the northern Transvaal. *S. Afr. J. Bot.* **58,** 268–74.

Oballa, P.O., 1993. Genetic variation within *Acacia karroo* Hayne. Oxford England, Unpubl. D. Phil. Oxford University, Oxford, UK.

Oballa, P.O. & P.A.S. Olngótie. 1992. *Acacia tortilis* (Forsk) Hayne: A study of genetic diversity and breeding systems. Unpub. D.Phil. Thesis, University of Oxford, Oxford, UK.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology & Evolution* **20**(9): 481 – 486.

Sankaran, M., Hanan, N.P., Scholes, R.J., Ratnam, J., Augustine, D.J., Cade, B.S., Gignoux, J., Higgins, S.I., Le Roux, X., Ludwig, F., Ardo, J., Banyikwa, F., Bronn, A., Bucini, G., Caylor, K.K., Coughenour, M.B., Diouf, A., Ekaya, W., Feral, C.J., February, E.C., Frost, P.G.H., Hiernaux, P., Hrabar, H., Metzger, K.L., Prins, H.H.T., Ringrose, S., Sea, W., Tews, J., Worden, J., Zambatis, N. 2005. Determinants of woody cover in African savannas. *Nature* **438**: 846-849.

Saunders, I.R., W.G. Bailey & J.D. Bowers. 1997. Evaporation regimes and evaporation modelling in an alpine tundra environment. *Journal of Hydrology* **195**: 99 – 113.

Schippers, P. & E. Jongejans. 2003. Release thresholds strongly determine the range of seed dispersal by wind. *Ecological Modelling* **185**(1): 93 – 103.

Schlaepfer, M.A., M.C. Runge & P.W. Sherman. 2002. Ecological and evolutionary traps. *Trends in Ecology & Evolution* **17**: 474 – 480.

Sharma, G.P. & K.J. Esler. 2008. Phenotypic plasticity among *Echium plantagineum* populations in different habitats of Western Cape, South Africa. *South African Journal of Botany* **74**(4): 746 – 749.

Shrestha, M.K., A. Golan-Goldhirsh & D. Ward. 2002. Population genetic structure in isolated populations of *Acacia raddiana* investigated by random amplified polymorphic DNA (RAPD) markers. *Biological Conservation* **108:** 119 – 127.

Smit, G.N. 2004. An approach to tree thinning to structure southern African savannas for long-term restoration from bush encroachment. *Journal of Environmental Management* **71**(2): 179 – 191.

Stanton, D.J., E.D. McArthur, D.C. Freeman & E.M. Golenburg. 2002. No genetic substructuring in *Artemisia* subgenus *Tridentatae* despite strong ecotypic subspecies selection. *Biochemical Systematics and Ecology* **30**(6): 579 – 593.

Storfer, A. 1996. Quantitative genetics: a promising approach for the assessment of genetic variation in endangered species. *Trends in Ecology & Evolution* **11**: 343 – 348.

Sultan, S.E. 2000. Phenotypic plasticity for plant development, function and life history.*Trends in Plant Science* **5**: 537 – 542.

Sultan, S.E. & S.C. Stearns. 2005. Environmentally contingent variation: phenotypic plasticity and norms of reaction. *Variation*:303 – 332.

Sweet, R.J. & G. Tacheba. 1985. Bush control with fire in semi-arid savanna in Botswana. *In* J.C. Tothill and J.C. Mott (eds). Ecology and management of the world's savannas. Australian Academy of Science. Canberra. pp 229 – 231.

Tews, J, A. Esther, S.J. Milton & F. Jeltsch. 2006. Linking a population model with an ecosystem model: Assessing the impact of land use and climate change on savanna shrub cover dynamics. *Ecological Modelling* **195**(3-4): 219 – 228.Titus, J.H., R.S. Nowak and S.D. Smith. 2002. Soil resource heterogeneity in the Mojave Desert. *Journal of Arid Environments* **52**: 269 – 292.

Van Auken, A.W. 2009. Causes and consequences of woody plant encroachment into western North American grasslands. *Journal of Environmental Management* **90**(10): 2931 – 2942.

Villeon L., van der Bank F. H.,Grobler J. P. & Wessels D. 2003. Allozyme variation in five populations of mopane, *Colophospermum mopane* (Fabaceae). *S. Afr. J. Bot.* **69,** 282–6.

Ward, D. 2005. Do we understand the causes of bush encroachment in African savannas? *African Journal of Range & Forage Science* **22**(2): 101 – 105.

Wendel, J.F. & N.F. Weeden. 1989. Visualization and Interpretation of Plant Isozymes. In: Soltis, D.E. & Soltis, P. (eds.). Isozymes in Plant Biology: Advances in plant science series. Vol. 4. Dioscorides Press, Oregon. pp. 5 – 45.

West-Eberhard, M.J. 2008. Phenotypic plasticity. *Encyclopedia of Ecology*:2701 – 2707.

Zida, D., L. Sawadogo, Tigabu, M., D. Tiveau & P.C. Oden. 2007. Dynamics of sapling population in savanna woodlands of Burkina Faso subjected to grazing, early fire and selective tree cutting for a decade. *Forest Ecology and Management* **243**(1): 102 – 115.

Chapter 4

WHAT IS THE ROLE OF LOCAL ADAPTATION AND PHENOTYPIC PLASTICITY IN TWO DIFFERENTIATING POPULATIONS OF THE BUSH-ENCROACHING SPECIES *Acacia mellifera* AT A LOCAL SCALE?

Abstract

We investigated local adaptation in two populations of *Acacia mellifera* near Pniel, a semi-arid savanna near Kimberley in the Northern Cape province of South Africa. One population appeared on rocky, andesitic laval ridges (pH_{KCL} 6.5-7; silt and clay ~24%) along the Vaal River, and displayed extensive encroachment and low stature. The other appeared in a sandveld area (soil pH_{KCL} 4, silt and clay ~16%) and was arranged in a clumped fashion, at low stem densities in tall *A. erioloba* savanna. Seeds were randomly sampled in each habitat for tests whether the two populations were, in fact, ecotypes, i.e. whether local adaptation exists in the two populations. Lime ($CaCO_3$) was used to simulate pH and cow-dung to manipulate the organic matter content in order to duplicate growing conditions of the two populations in a completely-crossed design. Detected interaction effects (between population source and pH; population source and organic matter and between pH and organic matter) and significant differences did not distinguish the two populations as the differences occurred across populations. Random genetic differences in the two populations, due to different environmental conditions, rather than local adaptation might thus be responsible for the observed phenotypic differences. Neither population differentiation nor local adaptation was thus established. It is likely that ecohydrological factors such as soil water holding capacity may play a role in determining observed biological and ecological differences between the two populations, which may be the result of phenotypic plasticity rather than local adaptation.

Key words: Acacia mellifera, *bush encroachment, population differentiation, local adaptation, lime, organic matter, vegetative reproduction and sexual reproduction.*

4.1 INTRODUCTION

Living organisms, both plants and animals, largely depend and interact with their environment such that their identity cannot be fully conceptualized if the significant role of their habitat is ignored. In this way, individuals reflect genotypes which compete well under local conditions, especially in terms of reproduction and survival (Lenormand, 2002). This observation emphasizes the role and magnitude of local adaptation in species' survival in their respective environments (Hufford & Mazer, 2003). Raven and Johnson (1992), states all cases of adaptation share the same fundamental characteristic: changes occur in the frequencies of alleles in populations, which alter the characteristics of the population and make it better adapted to its environment in which it is living. Therefore, it is the environment that dictates the direction and extent of the allelic frequency change (Carson, 1987). It is the genetic understanding of populations, which when considered in complement to field observations that provide most profound explanations and understandings of *why* and *how* natural phenomenons such as population differentiation, natural selection, reproductive isolation and allopathy occur. (Orr & Smith, 1998; Piertney *et al.* 1998). Landscape differences such as differential water or nutrient availability force species either to adapt to the local environmental conditions (by acting on locus viability) or be extinct and through local adaptation, population differentiation is manifested (Manel *et al.* 2003).

Shrestha *et al.* (2002) suggest that where population differentiation is evident, it remains a crucial conservation measure to treat each population as an individual entity, and conserve it, similar to individual species (the main reason for this conservation measure is that population differentiation and local adaptation could be supportive of speciation,

macroevolution and microevolution. However, although population differentiation, as governed by natural selection, might be evident, it still should be acknowledged that within a population there could be abundant polygenic variation or strong, stable selection such that if the population was exposed to new environmental conditions, suppressed genes might come into effect. Therefore the existence of a population does not guarantee non-dynamic stability - a population can be challenged by new environmental changes and be compelled to genetically adapt accordingly. These two conflicting observations are at the basis of quantitative genetics (Xu-Sheng & Hill, 2002). Quantitative genetics (the study of inheritance at the phenotypic level) has been recognized as one of the most promising fields which play an important role in unifying, understanding and making profound predictions about how a species or population might respond to environmental conditions (Steppan *et al.* 2002).

Environmental changes might be fixed, but different species found within that environment tend to respond differently to one particular change and the importance of understanding these different adaptive responses is becoming more recognized (Marshall *et al*. 1985; Schmid, 1985; Bennett & Grace, 1990). Such different adaptive responses have been noticed across taxa and between populations of a species (Schneiner & Goodnight, 1984; Blais & Lechowicz, 1989; Macdonald & Chinnappa, 1989). Where phenotypic differences have been correlated to the variation in resource availability, commonly genetic variability or plasticity, upon which natural selection acts, has been accepted as an adaptive measure (Chapin *et al*. 1987; Crick & Grime, 1987). Smekens and van Tienderen (2001), suggests all organisms respond to their environmental changes by plastic reactions in both morphology and physiology. They further propose that such reactions, which allow organisms to grow and reproduce under changing environmental conditions will always be favored by natural selection, *provided* genetic variation for those reactions is available *and* the costs do not outweigh the benefits of the response.

Two ecological populations of *Acacia mellifera* exist in the study area, in Kimberley in the Northern Cape. The two populations occurred in different habitats with the main, heavily encroaching population occurring on rocky andesitic laval ridges alongside the Vaal river. The second population was found in sporadic clusters in the flatter sandveld habitat, away from the river. The two habitats did not only differ in their level of rockiness and soil types but also differed considerably, particularly in pH, soil texture and cation exchange capacity. The rocky habitat (andesitic) had a neutral pH_{KCL} value (6.5-7.0) whereas the sandveld habitat was acidic with a pH_{KCL} of 4.0. Therefore the nutrient holding capacity of the andesitic soils was expected to be far greater than that of the sandveld. Due to higher fine fraction (clay and silt) in the soils, the rocky area may also have higher water retention ability. We endeavoured to determine the cause of observed biological and ecological differences between the two populations, which may play a role in the ability of the species to encroach, and thus in optimising management options. If the two populations are, indeed ecotypes, different management approaches may be considered, depending on the ecotype and interactions with the environment. In order to verify if the two populations were genetically distinct, it was thought necessary to establish if local adaptation had occurred. A completely-crossed design was set up with the notion that if seeds collected from one habitat could only survive in conditions similar to their original habitat that would imply local adaptation to that particular habitat (Raabova *et al*. 2007).

Whilst genetic differentiation might not be evident, local adaption which over time can lead to genetic drift, is also important in many ways. Differences in environmental conditions may require species to adapt to those conditions and as such certain genes that are active under one habitat may be inactive in another. This change in gene activity may manifest as different growth forms of the same species being evident (Hempson *et al*. 2007). Inability to acclimatize and adapt to an environment can easily lead to local extinction of a

species whilst adaption can lead to persistence and provide the species with opportunities to influence and be influenced by its new environment. A study by Hagos and Smit (2005) showed that the presence of *Acacia mellifera* subsp. *detinens* in an environment can have local benefits to the environment through nutrient cycling, especially nitrogen (nitrogen fixation), percentage of organic matter and Ca, with the highest values recorded in the area surrounding the stem base. P and K contents, and to a lesser extent Mg, were also higher under *A. mellifera* canopies. However, once a species has adapted to an environment, depending of some other factors such as fire regime, water and soil nutrient availability, and also the nature of the species in question, the species may end up outcompeting other species leading to compromised biodiversity at a local scale.

However, not all phenotypic changes may be indicative of differentiation or adaptation. According to Schlichting (1986), a plant's response has to be appropriate and be observable across different individuals when exposed to the same stimulus, to be considered adaptive. It has been reported that a number of plant traits are phenotypically plastic in response to resource levels that vary continuously (Orians *et al*. 2003). With phenotypic plasticity, it only implies that a species genetically reacts in a certain way when exposed to certain stimulus but once the stimulus is removed or altered, the species reacts in another way.

4.2 MATERIALS & METHODS

4.2.1 The Study Site

The area at Pniel (28^{o}35'126"**S**; 24^{o}32'248"**E**) near Kimberley, in the Northern Cape province of South Africa is typical of arid savanna showing encroachment of *Acacia mellifera.* Consequently sampling for this study was conducted here because bush encroachment by *A. mellifera* has been documented in the area and is of great concern to both conservationists

and land-use mangers in the area, (see Figure 4.2). There appeared to be two populations of *A. mellifera* in the study area. The main population occurred at very high densities and was found on rocky andesitic laval ridges alongside the Vaal River. A second population occurred sporadically in the sandveld away from the river. Bush encroachment was far more common in the rocky habitat. The soil types in the two habitats differed considerably, particularly in pH and cation exchange capacity. Andesite had a pH_{KCL} near neutral (6.5-7) while the sandveld had a pH_{KCL} of about 4. The rocky, andesitic soils also had relatively high clay content and high silt content (see Figure 4.1). Therefore the capacity of the andesitic soils to retain nutrients was considered greater than that of the sandveld, which had deep soils with no rocks and low silt content (see Figure 4.1). We predicted that there might be local adaptation to these vastly different soil types. Consequently, seeds were collected for greenhouse trials.

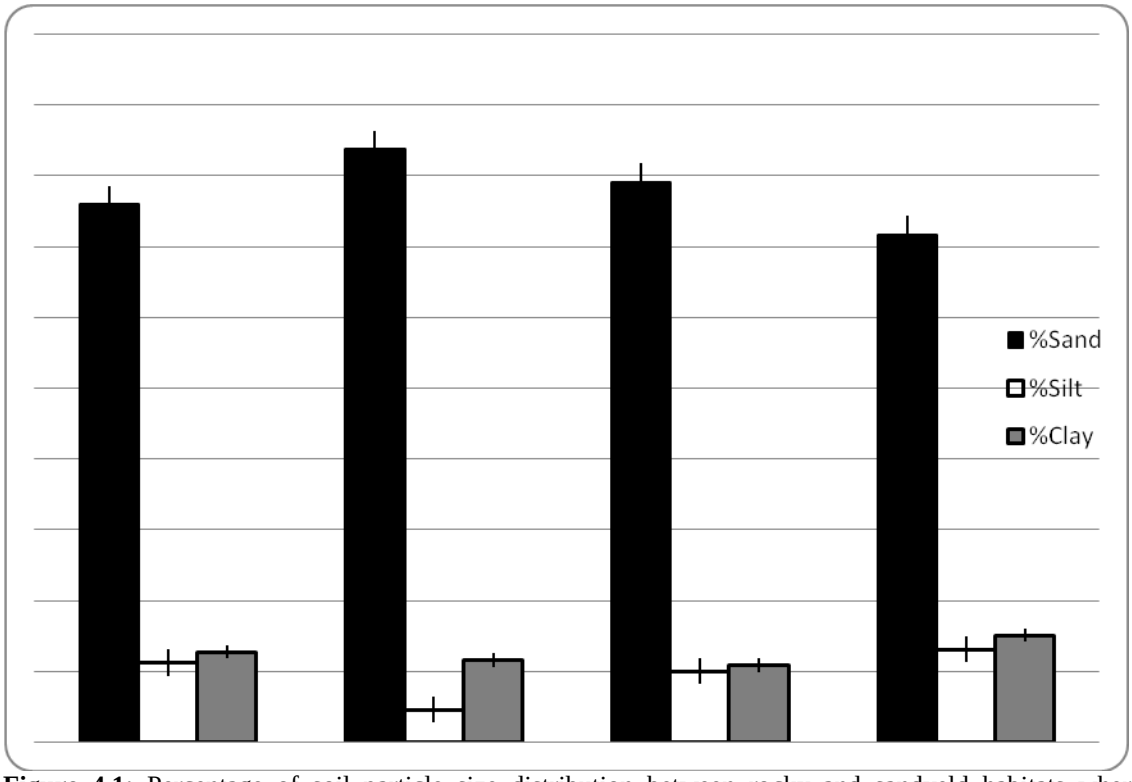

Figure 4.1: Percentage of soil particle size distribution between rocky and sandveld habitats where bush encroachment of *Acacia mellifera* subsp. *detinens* was observed.

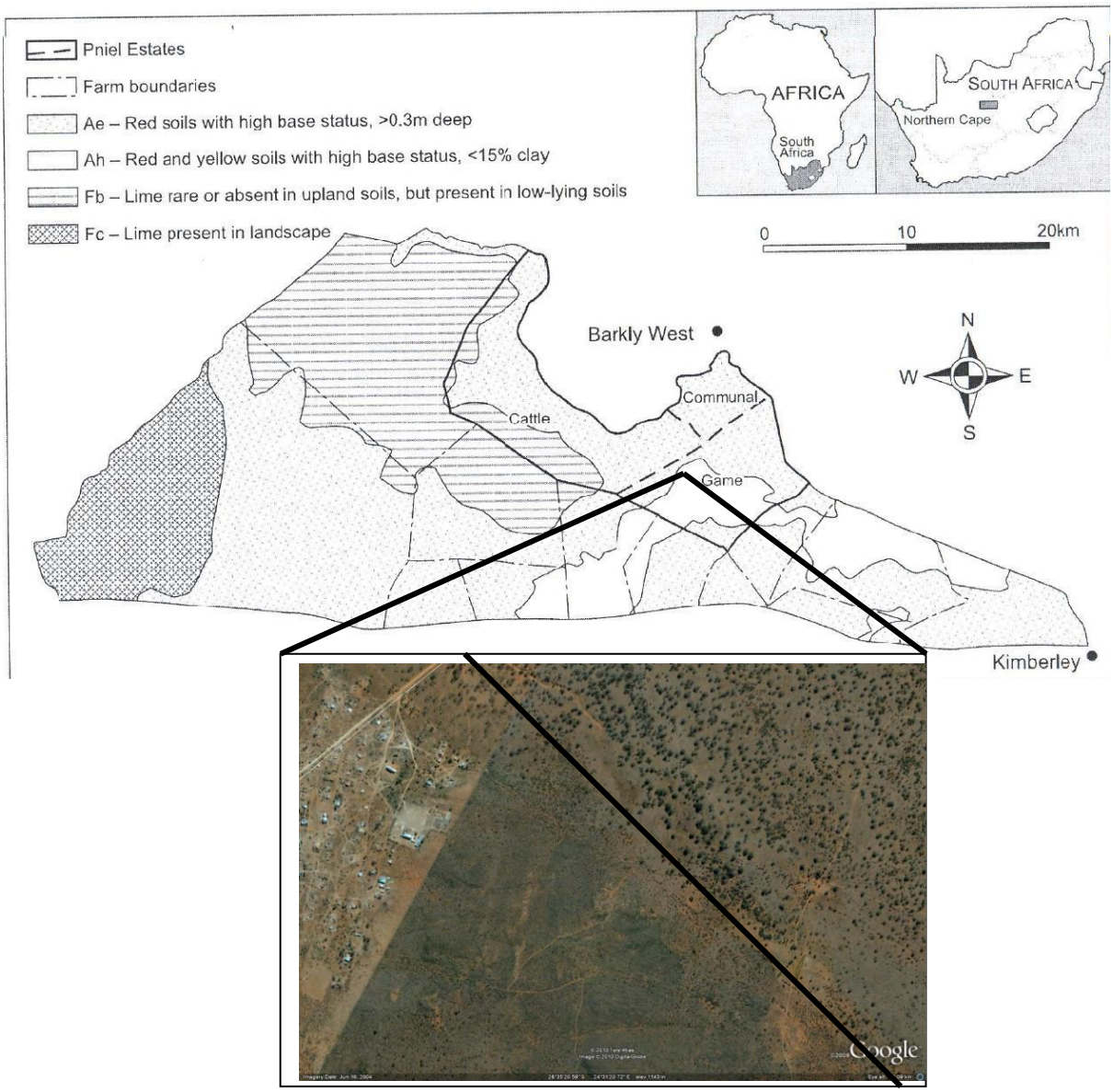

Figure 4.2: Map of the Pniel Estates, study site, and an insert of a Game Farm depicting two scenarios of bush encroachment by *Acacia mellifera* subsp. *detinens,* on either side of the diagonal line. On the top-right is bush encroachment in a sandveld area (encroachment appears in clusters within a grassland) and on the bottom-left, is encroachment on a rocky area (encroachment forms continuous carpets of *A. mellifera*). (Background image borrowed from Britz & Ward, 2007).

4.2.2 Seed Collection, Sand Treatment and Planting of Seeds

In each habitat type, (sandveld and rocky habitat), a total of 80 seeds of *A. mellifera* were randomly sampled, in the month of November, 2001. Seeds from green, live and viable pods were preferred over those in small, yellowish pods. Seeds were not frozen as those for electrophoretic analysis, rather they were kept in paper bags (as separate entities) and in a

cardboard box, at room temperature. In March 2002, these seeds were planted for germination in a greenhouse and after two months they were further transplanted into the correct treatments of lime ($CaCO_3$) and organic matter (cow-dung). No inoculation was deemed necessary in meeting the objectives and expectations of this experiment as nitrogen fixation or the lack thereoff was not deemed crucial to meeting objectives regarding local adaptation. For other objectives, when dealing with this particular species, nitrogen inoculation would strongly be recommended. The medium sand was bought from Malmesbury Sands, a mining company along the West Coast in the Western Cape province of South Africa. This sand was chosen on the basis of pH, as it "naturally" had a pH of 4.31. Thus, this was not treated with anything to simulate the sandveld pH of 4. In order to simulate the rocky habitat, however, the Malmesbury sand was treated with lime at 20g $CaCO_3$ per 1kg of Malmesbury sand.

The sand bought from Malmesbury, had an original pH of 4.31 and to determine how much lime ($CaCO_3$) had to be added in order to increase the pH to 7 (simulate the pH in the rocky area in Pniel), a standard pH curve was plotted. Increments of lime were added to a standard weight (2g) of sand and pH was measured. From this it was established how much lime ought to be added per unit weight of Malmesbury sand in order to achieve the required pH for the greenhouse experiment (completely-crossed design). A further 20g (2%) of cow-dung were added in every 1kg of Malmesbury sand in making a completely-crossed design in order to safeguard against threshold effect as no additional organic matter would be added during the course of the greenhouse experiment. Large, 5 litre pot-plant pots were used in this experiment and each pot carried 7.6kg of sand. The seedlings were then watered once every 3 days, as worked out from the annual rainfall reported in Pniel (400mm per annum).

4.2.3 Experimental Design

The greenhouse experiment was a completely-crossed design, with 80 *Acacia mellifera* seeds from each of the two habitats (sandveld and rocky area), planted in 4 plots wherein each plot had 20 replicates (Figure 4.2a). The experiment was run for nine months (February - October) after which the growth response was recorded. After 9 months, each seedling was examined to determine growth by measuring the following parameters; stem height, number of thorns, number of leaves, number of branches, mean root length and the number of lateral roots. The treatments were Source (rocky vs. sandy substrate), Lime (lime added vs. control) and Organic Matter (organic matter added vs. control). As this was a completely-crossed design, we analyzed these data using a full factorial ANOVA. Data were log-transformed prior to analysis because numbers were big.

Figure 4.2(a): *Acacia mellifera* collected from the study area were planted in a completely-crossed design, in the greenhouse. First they were grown in small pot-plant pots and then later transferred into 5-Litre pot-plant pots.

4.3 RESULTS

There was no significant effect of any of the experimental factors or their interactions on stem height (Table 4.1) or on the number of thorns (Table 4.2). However, a significant effect of lime addition on the mean root length was observed (F = 5.822, p = 0.017). It also appeared that roots were significantly longer when lime was added (Mean ± SE = 2.078 ± 0.015mm, n = 76), compared to the control (2.028 ± 0.016mm, n = 75) (data log-transformed).

Table 4.1: Effects of experimental factors on stem height (df = 1).

Factor	*SS*	*F*	*p*
Source	*0.003*	*0.079*	*0.778*
Lime	*0.004*	*0.126*	*0.723*
Organic Matter	*0.009*	*0.279*	*0.598*
Source x Lime	*0.000*	*0.003*	*0.960*
Source x Organic Matter	*0.009*	*0.286*	*0.593*
Lime x Organic Matter	*0.003*	*0.102*	*0.750*
Source x Lime x Organic Matter	*0.018*	*0.554*	*0.458*

Table 4.2: Effects of experimental factors on the number of thorns, defence mechanism (df = 1).

Factor	*SS*	*F*	*p*
Source	*0.048*	*1.435*	*0.233*
Lime	*0.014*	*0.414*	*0.521*
Organic Matter	*0.063*	*1.874*	*0.173*
Source x Lime	*0.003*	*0.102*	*0.749*
Source x Organic Matter	*0.027*	*0.803*	*0.372*
Lime x Organic Matter	*0.005*	*0.156*	*0.693*
Source x Lime x Organic Matter	*0.000*	*0.006*	*0.938*

No significant effect of any other factor or their interaction on mean root length was observed except for lime (Table 4.3 and Fig. 4.3). Another significant effect of lime addition was observed on the number of leaves as opposed to when lime was not added, (Mean ± SE number of leaves with lime addition = 1.031 ± 0.092, n = 76; without lime = 0.080 ± 0.039, n = 75). With the addition of lime, the number of branches also increased significantly compared to when lime was not added, (Mean ± SE number of branches with lime addition = 0.676 ± 0.046, n = 76).

Table 4.3: Effects of experimental factors on mean root length, as a function of water and soil nutrient access (df = 1).

Factor	*SS*	*F*	*p*
Source	*0.057*	*3.235*	*0.070*
Lime	***0.102***	***5.822***	***0.017***
Organic Matter	*0.000*	*0.025*	*0.875*
Source x Lime	*0.004*	*0.208*	*0.649*
Source x Organic Matter	*0.041*	*2.333*	*0.129*
Lime x Organic Matter	*0.007*	*0.398*	*0.529*
Source x Lime x Organic Matter	*0.019*	*1.112*	*0.293*

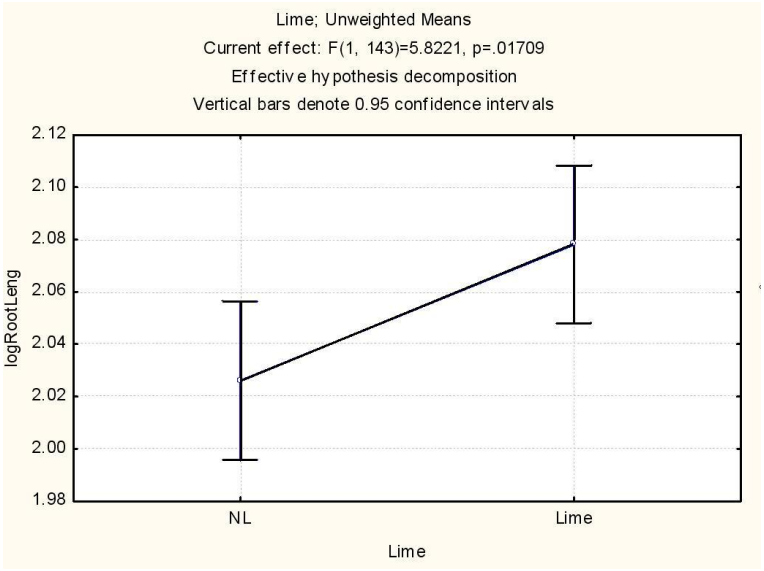

Figure 4.3: Lime addition, as a factor influencing soil pH, showed a significant effect on root length. NB: Addition of lime was done to simulate the rocky habitat pH conditions and no lime (NL) signified the sandveld.

With regard to source, a significant effect on the number of lateral roots produced was observed (F = 4.016, p = 0.047), see Fig.4.4. Significantly more roots were produced when lime was added (Mean ± SE number of lateral roots from the rocky substrate = 0.972 ± 0.036, n = 73; from the sandy substrate = 0.860 ± 0.040, n = 78). None of the other factors or their interactions had a significant effect on lateral root production, although the effect of organic matter was marginally non-significant (Table 4.4).

Table 4.4: Effects of experimental factors on lateral roots (df = 1).

Factor	*SS*	*F*	*p*
Source	***0.428***	***4.016***	***0.047***
Lime	*0.201*	*1.883*	*0.172*
Organic Matter	*0.386*	*3.628*	*0.059*
Source x Lime	*0.000*	*0.002*	*0.963*
Source x Organic Matter	*0.024*	*0.221*	*0.639*
Lime x Organic Matter	*0.052*	*0.489*	*0.486*
Source x Lime x Organic Matter	*0.299*	*2.808*	*0.096*

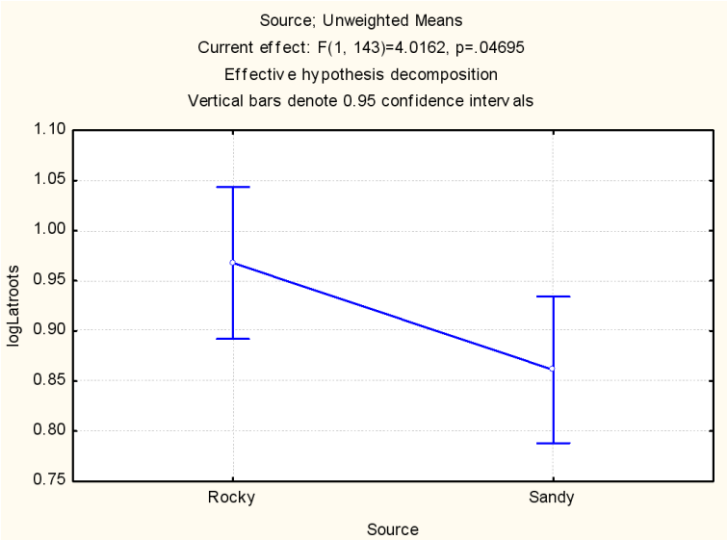

Figure 4.4: Seed source showed a significant effect on lateral roots.

Similar significant effects of source and lime addition were also observed on the number of leaves and number of branches (Table 4.5 & 4.6, respectively) also see Figs. 4.5(a), 4.5(b), 4.5(c) and Figs. 4.6(a), 4.6(b), 4.6(c), respectively. Mean ± SE number of leaves in plants from the rocky habitat was 0.334 ± 0.069, n = 73 and 0.768 ± 0.099, n = 78 in plants from the sandy substrate. Similar plants from the sandy habitat had significantly more branches than plants from the rocky habitat (Mean ± SE number of branches from the sandy substrate = 0.640 ± 0.044, n = 78; from the rocky substrate = 0.411 ± 0.040, n = 73).

Table 4.5: Effects of source and lime addition on the number of leaves produced (df = 1).

Factor	*SS*	*F*	*p*
Source	***8.040***	***33.000***	***< 0.001***
Lime	***33.230***	***136.200***	***< 0.001***
Organic Matter	*0.120*	*0.500*	*0.487*
Source x Lime	***13.360***	***54.800***	***< 0.001***
Source x Organic Matter	*0.050*	*0.200*	*0.649*
Lime x Organic Matter	*0.300*	*1.200*	*0.268*
Source x Lime x Organic Matter	*0.450*	*1.800*	*0.178*

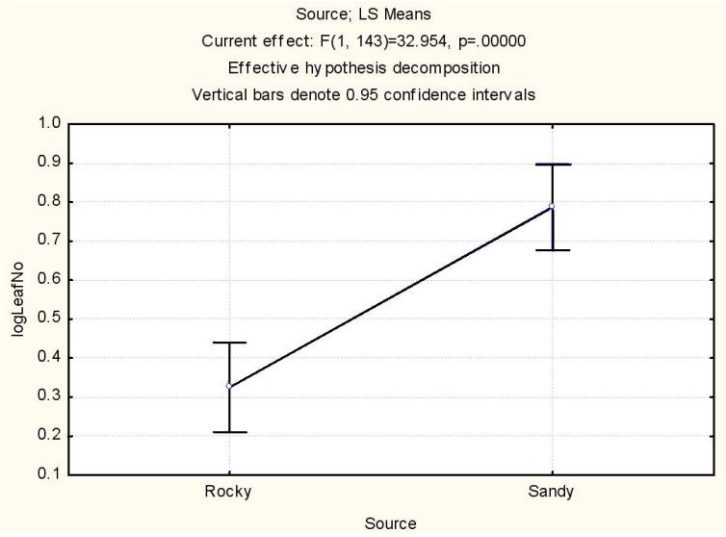

Figure 4.5(a): Seed source had a significant effect on the number of leaves produced by seedlings.

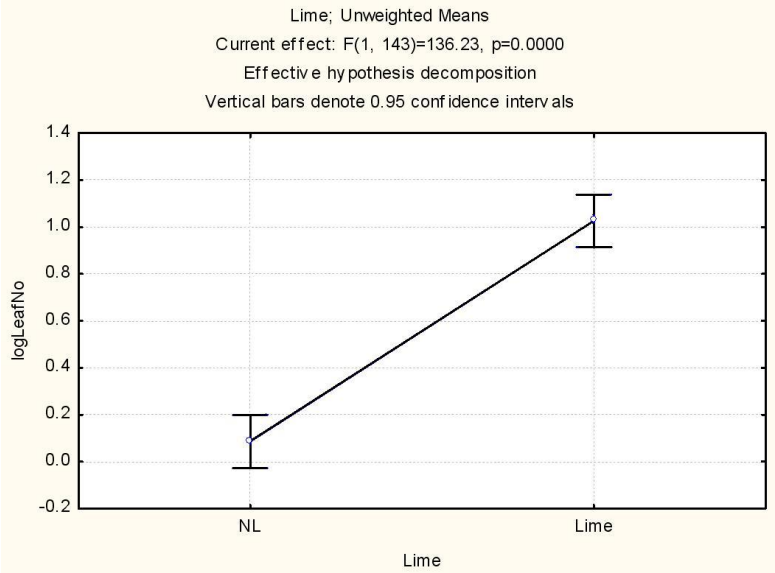

Figure 4.5(b): The addition of lime to simulate rocky habitats in terms of pH, where the exclusion of lime (NL) simulated sandy soils of the study area, also had a significant effect on the number of leaves produced by *Acacia mellifera* seedlings.

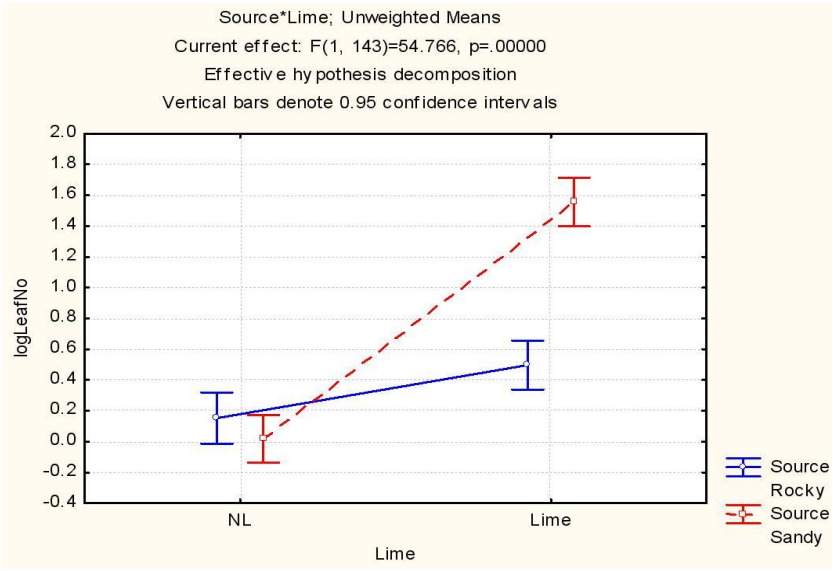

Figure 4.5(c): The combination of source and soil pH, as defined by the addition/exclusion of lime, also had a significant effect of the number of leaves produced by *Acacia mellifera* seedlings.

Table 4.6: Effects of source and lime addition on the number of branches (df was 1).

Factor	*SS*	*F*	*p*
Source	***2.171***	***20.240***	***< 0.001***
Lime	***3.260***	***30.390***	***< 0.001***
Organic Matter	*0.031*	*0.290*	*0.592*
Source x Lime	***1.290***	***12.030***	***0.001***
Source x Organic Matter	*0.130*	*1.220*	*0.272*
Lime x Organic Matter	*0.089*	*0.830*	*0.364*
Source x Lime x Organic Matter	*0.013*	*0.120*	*0.731*

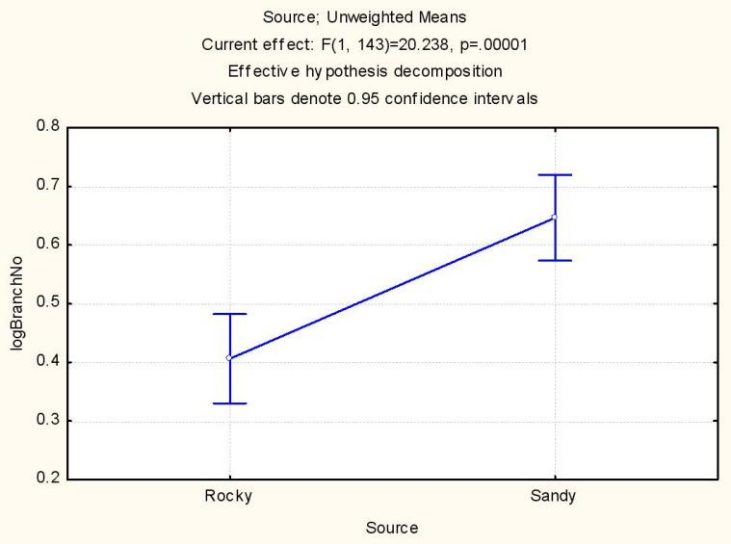

Figure 4.6(a): Seed source reflected a significant effect on the number of branches formed by *Acacia mellifera* seedlings in the greenhouse experiment.

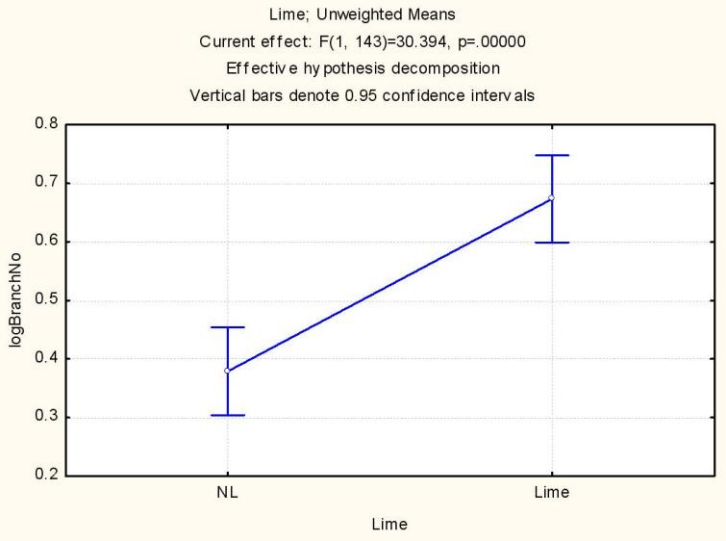

Figure 4.6(b): The addition of lime to simulate rocky habitats in terms of pH, where the exclusion of lime (NL) simulated sandy soils of the study area, also had a significant effect on the number of branches produced by *Acacia mellifera* seedlings.

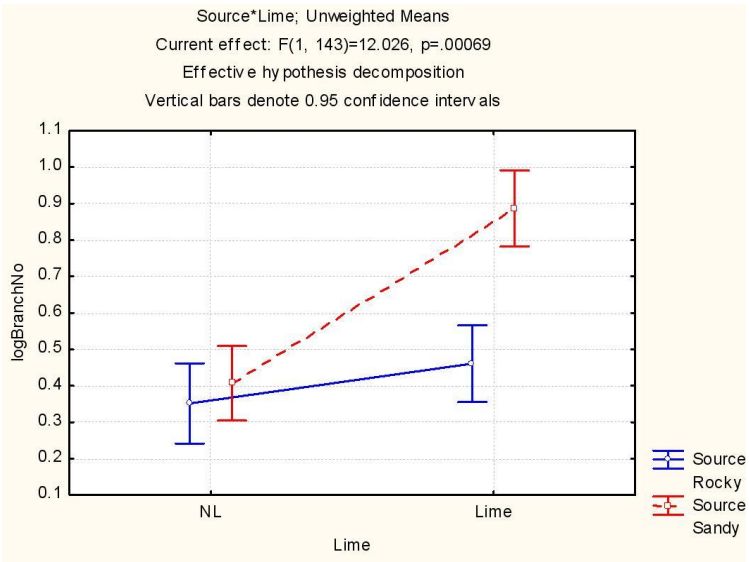

Figure 4.6(c): The combination of source and soil pH, as defined by the addition/exclusion of lime, also had a significant effect of the number of branches formed by *Acacia mellifera* seedlings.

In both cases there was a significant interaction effect between lime and source due to the increase in leaf and branch numbers caused by lime addition in plants from the sandy substrate (Figs. 4.1 & 4.2). There was virtually no change in these traits with lime addition in plants from the rocky substrate.

4.4 DISCUSSION

Detection of any significant interaction effect between population source and soil treatment in the greenhouse experiment would constitute proof of local adaptation. Local adaptation, in turn would imply that a sandveld population could not do as well in the rocky habitat as in the sandveld, and vice versa. This then, would prove that population differentiation is most likely to have taken place as these observed populations have been around for decades and that is would imply that the two populations of *Acacia mellifera* which were observed in Pniel (study area), were genetically different or else it could merely be simple phenotypic plasticity as was found to be the case with *Acacia karroo* growth form populations occurring in distinct environments (Mboumba & Ward, 2008).

After nine months (February - October) of growth monitoring of seedling grown from seeds from the two sources (sandveld and rocky habitat) in a completely-crossed design with lime ($CaCO_3$) and organic matter (cow dung) as soil treatments, no significant difference were observed. Non-significant results were observed in terms of stem height, number of thorns, number of leaves produced, number of petioles, root length and number of lateral roots which were measured at the end of the nine-month period. Thus, on the basis of the greenhouse experiment, it could be concluded that no strong evidence for population differentiation was evident. This finding is unlike the observations made by Shrestha *et al.* (2002), in their study on *Acacia raddiana* where they found that western Negev and Arava valley populations of the Negev desert in Israel were highly differentiated. The observed significant differences and interaction effects (between population source and pH; population source and organic matter and between pH and organic matter), could thus be results of random genetic differences yielding phenotypic differences probably as a result of soil particle differences in terms of clay and silt contents and also water holding capacities of the two habitats (Makholela *et al*. 2003). Divergent growth forms of the same species have been

observed in other species growing in different environmental conditions such as the study on *Colophospermum mopane* in Kruger National Park, South Africa (Hempson *et al*. 2007) and also on genetic variation of two *Acacia karroo* populations (Mboumba & Ward, 2008) from extreme environments in South Africa: arid Karoo (Leeu Gamka) and wet coastal forest (Richards Bay). The results obtained in this study, through molecular genetics and greenhouse experiments firstly complemented each other in confirming no population differentiation and no local adaption and both these result indicate that the observed population were in fact a single population. This finding is in line with similar observation made elsewhere, in other species, where different growth forms were also observed and it was postulated that population differentiation might have taken place but molecular genetics disproved such postulations (Hempson *et al.* 2007; Mboumba & Ward, 2008). Interestingly, these similar findings, on *C. mopane* (Hempson *et al.* 2007) and on *A. karroo* (Mboumba & Ward, 2008) were also on savanna systems. Therefore, a pattern is emerging that savanna species can exist in different growth forms but still remain members of the same population and this highlights the role of polygenic variation in these species (Shrestha *et al.* 2002). Therefore, had nitrogen inoculation been carried out in this experiment, it would in all likelihood not have yielded different results than found in this study. So, whilst nitrogen inoculation is essential since this species nodulates, in addressing the objectives of this study, inoculation might not have been essential.

With regards to vegetative and sexual reproduction modes, a plant might find it difficult to propagate vegetatively in rocky areas just because of the density of rocks impeding root extension. Through sexual reproduction the plant may ensure that its offspring will not grow in such a stressful environment as itself (Munkert, 2009). Dispersing seeds to other places further away from a undesirable environme ı achieve this escape from a stressful

environment as post-dispersal seed dispersal is acknowledged as one of the major presumed advantages of seed dispersal (Barbera *et al.* 2006).

4.5 CONCLUSSION

In spite of the fact that there were some signs of population differentiation between the rocky and sandveld populations of *A. mellifera*, e.g. with regard to petioles, root length etc., there was no solid and clear-cut differentiation observed. Interaction effects (between population source and pH; population source and organic matter and between pH and organic matter) and significant differences were observed, however, none were consistent with population differentiation of the two populations as the differences occurred across populations. All this then did not lay a conclusive foundation to say the two populations had genetically differentiated nor to say there was local adaptation. Thus the two populations considered in this study might not be genetically different; rather phenotypic plasticity came through as a result of soil texture in the different habitats, water availability and soil pH. These phenotypic differences might merely be responses of temporary adaptation by the populations to their respective environmental factors. The fact that no local adaptation was detected indicates that the genetic responses to these different habitats were as a result of flexible genetic plasticity (Schlichting, 1986).

4.6 ACKNOWLEDGEMENTS

Glory be to the Heavenly Father for guiding, protecting and giving me the strength to further my education, especially after my road accident whilst at the University of Stellenbosch. I sincerely would like to thank the staff and students, of the years 2001 – 2002, of the Conservation Ecology department of the University of Stellenbosch for their support and making my academic study at the University o nbosch such a stimulating and awarding

time. My supervisor Dr. Shayne Jacobs for his commitment, encouragement and all that he taught me. Professor David Ward of the University of KwaZulu-Natal for instilling in me the interest in savanna systems. Professor Colleen Downs of the University of KwaZulu-Natal (Pietermaritzburg campus) for her immeasurable assistance with my write-up. I would like to thank Nozuko Booi and Minette Naude for their assistance in my soil sample analysis. To Vernon of the Malmesbery sand mines for sponsoring my project with sand. To the National Research Foundation (NRF), Cape Tercentenary, Erickssen & Ethel Trust Foundation and the University of Stellenbosch for financial support.

4.7 LITERATURE CITED

Barbera, G.G., J.A. Navarro-Cano & V.M. Castillo. 2006. Seedling recruitment in a semi-arid steppe: The role of microsite and post-dispersal seed predation. *Journal of Arid Environments* **67**(4): 701 – 714.

Bennett, B.D. & J.B. Grace. 1990. Shade tolerance and its effect on segregation of two species of Louisiana *Iris* and their hybrids. *American Journal of Botany* **77:** 100 – 107.

Blais, P.A. & M.J. Lechowicz. 1989. Variation among populations of *Xanthium strumarium* (Compositae) from natural and ruderal habitats. *American Journal of Botany* **76:** 901 – 908

Britz, M-L & D. Ward. 2007. Dynamics of woody vegetation in a semi-arid savanna, with a focus on bush encroachment. *African Journal of Range & Forage Science* **24**(3): 131 – 140.

Carson, H.L. 1987. The process whereby species originate. *Bioscience* **37**: 715 – 720.

Chapin, III F.S., A.J. Bloom, C.B. Field and R.H. Waring. 1987. Plant responses to multiple environmental factors. *Bioscience* **37**: 49 – 57.

Crick, J.C. & J.P. Grime. 1987. Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasted ecology. *New Phytologist* **107**: 403 – 414.

Hagos, M.G. & G.N. Smit. 2005. Soil enrichment by *Acacia mellifera* subsp. *detinens* on nutrient poor sandy soils in a semi-arid southern African savanna. *Journal of Arid Environments* **61**(1): 47 – 59.

Hempson, G.P., E.C. February & A.G. Verboom. 2007. Determinants of savanna vegetation structure: Insights from *Colophospermum mopane. Austral Ecology* **32**, 429 – 435.

Hufford, K.M. & S.J. Mazer. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution* **18**: 147 – 155.

Lenormand, T. 2002. Gene flow and the limits to natural selection. *Trends in Ecology & Evolution* **17**: 183 – 189.

Makholela, T., H. van der Bank & K. Balkwill. 2003. A preliminary sduty of allozyme variation in three rare and restricted endemic *Barleria greenii* (Acanthaceae) populations. *Biochemical Systematics and Ecology* **31**(2): 141 – 154.

Manel, S., M.K. Schwartz, G. Luikart & P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution* **18**(4): 189 – 197.

Marshall, D.L., N. Fowler & D.A. Levin. 1985. Plasticity in yield components in natural populations of three species of *Sesbania. Ecology* **66**: 753 – 761.

Mboumba, G.B. & D. Ward. 2008. Phenotypic plasticity and local adaptation in extreme populations of *Acacia karroo. African Journal of Range and Forage Science* **25:** 121 – 130.

McDonald, S.E. & C.C. Chinnappa. 1989. Population differentiation for phenotypic plasticity in the *Stellaria longipes* complex: comparisons among cytotypes and habitats. *Evolution* **42**: 1036 – 1046.

Munkert, H.C. 2009. Sexual and vegetative regeneration of three leguminous tree species in South African savannas. *South African Journal of Botany* **75:** 606 – 610.

Orians, C.N., S. Lower, R.S. Fritz & B.M. Roche. 2003. The effects of plant genetic variation and soil nutrients on secondary chemistry and growth in shrubby willow, *Salix sericea*: patterns and constraints on the evolution of resistance traits. *Biochemical Systematics & Ecology* **31**: 233 – 247.

Orr, M.R. & T.B. Smith. 1998. Ecology and speciation. *Trends in Ecology & Evolution* **13**: 502 – 265.

Piertney, S.B., A.D. MacColl, P.J. Bacon & J.F. Dallas. 1998. Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology* **7**: 1645 – 1654.

Raabova, J., Z. Munzbergova & M. Fischer. 2007. Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus. Biological Conservation* **139**(3-4): 348 – 357.

Raven, P.H. & G.B. Johnson. 1992. Biology, 3rd ed. Mosby Year Book. USA. pp 389 –390.

Schneiner, S.M. & C.J. Goodnight. 1984. The comparison of phenotypic plasticity and genetic variation in populations of the grass *Danthonia spicata*. *Evolution* **38**: 845 – 855.

Schlichting, C.D. 1986. The evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **17**: 667 – 693.

Schmid, B. 1985. Clonal growth in grassland perennials. III. Genetic variation and plasticity between and within populations of *Bellis perennis* and *Prunella vulgaris. Journal of Ecology* **73:** 819 – 830.

Shrestha, M.K., A. Golan-Goldhirsh & D. Ward. 2002. Population genetic structure and the conservation of isolated populations of *Acacia raddiana* in the Negev Desert. *Biological Conservation* **108**: 119 – 127.

Smekens, M.J. & P.H. van Tienderen. 2001. Genetic variation and plasticity of *Pantago coronopus* under saline conditions. *Acta Ocologia* **22**:187 – 200.

Steppan, S.J., P.C. Phillips & D. Houle. 2002. Comparative quantitative genetics: evolution of the G-matrix. *Trends in Ecology & Evolution* **17**: 320 – 327.

Xu-Sheng, Z. & W.G. Hill. 2002. Joint effects of pleiotropic selection and stabilizing selection on the maintenance of quantitative genetic variation at mutation-selection balance. *Genetics* **162**: 459 – 471.

Chapter 5

OVERALL CONCLUSION

Two populations of *Acacia mellifera* were noted in Pniel, near Kimberley in the Northern Cape province of South Africa. The two populations occurred on different habitats. One occurred on andesitic, laval ridges along the Vaal River and these areas are predominantly rocky. The other grows in a neighbouring sandveld area. The two habitats appeared to be different in soil pH and soil texture in that the rocky had a relatively high clay content and significantly high silt content. As a result water retention might be higher in the rocky areas than in the sandveld where water and soil nutrient could even easily leach through the soil to the water table. Seeds were collected from the two habitats for allozyme analysis and attempting to separate the two populations on Principal Coordinate analysis (PCoA) showed no significant differences. The performed Mantel test and the Two-sampled T-test also showed non-significant results confirming the two observed populations have not genetically differentiated.

The presence of rocks in the rocky areas, in combination with high clay and silt contents, might well be contributive to higher water holding capacity of this habitat (see e.g. Mackay, 2001). This capacity might be essential in making water available for longer time periods, as opposed to the sandveld where there were was significantly low silt, low clay and no rocks. The near neutral soil pH_{KCL} (around pH 7) in the rocky areas could be essential in maintaining soil nutrients in useable forms as opposed to the sandveld where the soil pH_{KCL} was very acidic, around 4 (van Asten, 2003).

The rocks, in the rocky area, could also restrict root extension by forming rock pockets. This would thus limit access to soil nutrients, at a later stage in a plant's life which might lead to a plant becoming "dwarfed" as it grows older. If this is probable, plants should then be healthy

while young as they should have less interrupted access to soil nutrients and their growth status or rate should change as they grow older when they have exhausted nutrients around them (Bouillet *et al*. 2002). The rocky populations showed signs of interrupted access to nutrients. They all were of roughly the same height ("dwarfed"), had fewer seedpods and their leaves were not as green as those of the sandveld populations.

The high density of "dwarfed" individuals in the rocky area could be reflecting a possibility that plants were once plentiful in the area because of resource abundance (Tschirhart, 2002). That is, probably nutrients were once plenty and water was not limiting, thus plants grew well. But, at a later stage, either nutrients or water became limiting and growth got negatively affected. In the sandveld, subsurface rock pockets should be non-existent and access to nutrients not affected. Plants in the sandveld appeared much taller and greener, with many more seedpods than their counterparts in the rocky areas.

After the analysis of the levels of genetic similarity/allozymic variability, it appeared that the overall direction of spread was toward the east. Although the seed dispersal vector was not ascertained, it is likely that winds might be influential in determining the direction of spread, as opposed to animals or any other vector. Knowing the direction of spread might be helpful in predicting which other area is likely to be encroached in the near future and who should not worry about their land being invaded by *A. mellifera.*

Finally, a greenhouse experiment was conducted where a completely-crossed design was set up. Seeds were collected from both habitats and planted in different plots with different treatment to simulate the two habitats. Little evidence to show the two populations are significantly different was collected. In spite of the fact that there were some signs of population differentiation between the rocky and sandveld populations of *A. mellifera,* e.g. with regard to petioles, root length etc., there was no clear differentiation. The detected differentiation was not consistent with genetic differentiation between the two populations

and the observed significant differences occurred across populations. Measuring the genetic distance between and within the two sampled habitats showed no sign of a genetic gap between the two populations. All this then did not lay a conclusive foundation to say the two populations had differentiated nor to say there was local adaptation. Thus the two populations considered in this study, were not genetically different, rather the different natures of their environments as a driving force behind phenotypic plasticity might be responsible for the observed growth forms. This therefore could imply the genetic responses that led to the observed growth forms, were not fixed.

My results are important in that they reflect the value of understanding genetic profiles of both species and populations in order to adequately manage biodiversity features. Management based at macro-molecular level will work but the findings of this study clearly show that we could undermine basic principles of diversity and as a result we might lose genetic diversity. Genetic comprehension of biodiversity provides well informed decision-making, otherwise phenotypically observed phenomena (i.e. evident to the naked eye), cannot be fully understood or managed. This study shows we need to investigate even the local environmental differences such as soil profiles, quantification of clay and silt contents in the environments. Certain species can regenerate both vegetatively and sexually and this study has shown the significance of attempting to understand under what conditions a species will engage in whichever mode of reproduction. This can inform recommendations on how to control the spread of such a species, what control measures to apply (mechanical, chemical or biological controls). Lastly, establishing the direction of a problem is crucial in prioritizing where and when to implement an adaptive or reactive strategy and where to direct funding. For farmers, both commercial and communal, bush encroachment is a major concern as the ability of the land to sustain farming gets reduced. Therefore such knowledge could be of great value to them and other land-use managers such as conservation agencies.

5.1 LITERATURE CITED

Bouillet, J., J. Laclau, M. Arnaud, A.T. M'Bou, L. Sainth-Andre & C. Jourdan. 2002.Changes with age in the spatial distribution of roots of *Eucalyptus* clone in Congo: Impact on water and nutrient uptake. *Forest, Ecology & Management* **171**: 43 – 57.

Mackay, D.S. 2001. Evaluation of hydrologic equilibrium in a mountainous watershed: incorporating forest canopy spatial adjustment to soil biogeochemical processes. *Advances in Water Research* **24**: 1211 – 1227.

Tschirhart, J. 2002. Resource competition among plants: from maximizing individuals to community structure. *Ecological Modelling* **148**: 191 – 212.

van Asten, P.J.A., L. Barbiero, M.C.S. Wopereis, J.L. Maeght & S.E.A.T.M. van der Zee. 2003. Actual and potential salt-related soil degradation in an irrigated rice scheme in the Sahelian zone of Mauritania. *Agricultural Water Management* **60**: – 32.

APPENDIX I (Stocks Used)

Agar was used to form the gel medium, when mixed with TRIS used for running the allozyme extractions. The MTT and PMS were used to form the staining dye that will colour the allozyme fragments so they become visible in the gel.

AGAR GEL (2%):

Weigh 2g agar
Add 110ml dH_2O
Swirl
Bring to boil on hotplate – watch it very carefully!
Place in water bath, until use

MTT: (0.2%)

(Dimethylthiazol-2-yl Diphenyltetrazolium bromide) CARCINOGENIC
Weigh 0.2g MTT
Add 100ml dH_2O
Mix properly and keep refrigerated

PMS: (0.1%)

(Phenazine Methosulphate) CARCINOGENIC!!
Weigh 0.1g PMS
Add 100ml dH_2O
Mix properly and keep refrigerated

TRIS: (0.1M)

Weigh 6.06g Tris
Add 500ml dH_2O
Mix thoroughly and keep refrigerated

BUFFERS:

Buffers were mediums used for carrying the electric current and facilitate the movement banding patterns through the gels.

A. Ridgeway buffer (RW)

Electrode (pH 8.0)

0.06M Lithium Hydroxide	25.20g
0.3M Boric Acid	185.50g

Add 2 liters dH_2O

Adjust pH to 8.0 with NaOH / HCl

Make up to final volume of 10 liters with dH_2O

Gel (pH 8.7)

0.03M Tris	36.34g
0.005M Citric Acid	10.51g
RW Electrode buffer	100ml

Add about 850ml dH_2O

Adjust pH to 8.7 with NaOH / HCl

Make up to a final volume of 1 liter with dH_2O

To use: Dilute 33ml RW Gel buffer with dH_2O to 330ml

B. TBE buffer

Electrode & Gel (pH 9.0)

0.087M Tris	105.30g
0.087M Boric Acid	5.40g
0.001M EDTA	3.70g

Add 2 liters dH_2O

Adjust pH to 9.0 with NaOH / HCl

Make up to a final volume of 5 liters with dH_2O

To use: *Electrode;* 500ml undiluted

Gel buffer; 330ml undiluted

C. TC buffer

Electrode & Gel (pH 6.9)

0.15M Tris	181.05g
0.05M Citric Acid	105.00g

Add 2 liters dH_2O

Adjust pH 6.9 with NaOH / HCl

Make up to a final volume of 5 liters with dH_2O

To use: *Electrode;* 330ml undiluted

Gel buffer; dilute 11ml TC Electrode with dH_2O to 330ml

APPENDIX II (Sample, Gel & Enzyme Preparation)

This section details the preparation of allozyme extracts from seeds, the preparation of gels for running the extracts through the different buffers. Enzymes preparations were used to excise protein fragments from the allozyme extracts. Different enzymes will recognize and cut different fragments.

Sample Preparation

Label Eppindorf tubes, as required so can identify in future

Remove seed from seedpod

Dissect cotyledon from seed coat

Put on glass sauce and slice into small slices with sharp scalpel

Put in appropriate Eppindorf and add about 0.2g of sand {0.1 – 0.3mm (50 – 150 mesh), purified by acid}

Add 100ul of 0.1M Tris and homogenize with a rotating glass rod, into a fine suspension

Keep samples in ultra deep-freezer (-80^{o}C)

Gel Preparation

Prepare perspex gel molds.

Weigh 42g starch and place in 1l Erlenmeyer flask.

Place 230ml of appropriate gel buffer into another Erlenmeyer flask and bring to boil on hotplate.

Add the remaining 100ml of gel buffer into flask containing starch.

Make a homogenous slurry by swirling flask until starch is well emulsified

Once the 230ml buffer is boiling, add to the starch slurry quickly while swirling flask as you add.

Heat the starch solution on hotplate again, while swirling occasionally, until solution becomes clear.

Remove from hotplate and degas to remove air bubbles.

Pour the gel onto perspex gel mold, allow to cool, cover with plastic wrap to prevent evaporation and store in fridge.

Enzyme Preparation

Glucose-6-phosphate dehydrogenase **(G6PDH)**

10 ml Tris-8, 0.1M

20 mg glucose-6-phosphate

10 mg NADP

10 mg $MgCl_2$

2 ml MTT

250 ul PMS

10ml melted agar (2%)

Incubate at 40^oC in dark.

Glucose-6-phosphate isomerase **(PGI)**

10 ml Tris-8, 0.1M

10 mg Frucose-6-phosphate

10 mg NAD

s glucose-6-phosphate dehydrogenase

2 ml MTT

250 ul PMS

10 ml melted agar (2%)

Incubate at 40^oC in dark.

Peptidase-L-leucylglycylglycine **(PEP-LGG)**

10 ml Tris-8, 0.5M

20 mg L-leucylglycyleglycine (LGG)

10 mg Snake Venom (TOXIC –amino acid oxidase)

100 u Peroxidase

30 mg O-Dianisidine TOXIC

10 ml melted agar (2%)

Incubate at 40^{o}C.

Fructose biphosphate aldolase **(FBA)**

10 ml Tris-8, 0.1M

20 mg Fructose-1,6-diphosphate

40 mg Arsenate

20 mg NAD

10 units Glyceraldehyde-P-dehydrogenase

2 ml MTT

250 ul PMS

Incubate at 40^{o}C in dark.

Phosphoglucomutase **(PGM)**

10 ml Tris-8,0.1M

20 mg Glucose-1-phosphate

10 mg NADP

10 mg $MgCl_2$

2 ml MTT

250 ul melted agar (2%)

Incubate at 40^{o}C in dark.

Diaphorase, (cytochrome b5 reductase) **(DIA)**

10 ml Tris-8, 0.1M

10 mg NADH

5mg Dichlorophenol-indophenol

2 ml MTT

NO PMS!

Incubate at 40^{o}C in dark.

APPENDIX III (Greenhouse Experiment)

The big 5-liter pot-plant pots were used for planting seedlings once the seedlings had germinated and these pots had enough depth to allow for free expansion and growth of roots. Malmesbery sand was used as the growth medium as opposed to transporting sand from the study area. Malmesbery had natural pH close to that of the sandveld, in the study area and hence it was an ideal growth medium to use as it was available very close to the university. Lime was used to increase the Malmesbery sand pH in order to simulate the rocky habitat pH. A calibration curve was plotted in order to determine how much lime to add to a unit volume of sand to acquire the rocky habitat pH_{KCL} of 7.0. Organic matter (cow-dung) was added to the Malmesbery sand to duplicate the organic matter content of the study area and provide soil nutrients for the planted seeds.

Item(s)	*Quantity*
Big 5 liter pot-plant pots	160
Malmesbery sand	7.6kg per pot
Lime ($CaCO_3$)	$0.2x10^{-4}$g per unit gram of sand
Organic matter (cow-dung)	$0.2x10^{-4}$g per unit gram of sand